Mushrooms, Snacks, and Dairy Foods: Health Impacts, Consumption Patterns, and Dietary Guidance

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Dedication

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Abstract

The Dietary Guidelines for Americans (DGA) are designed to incorporate current scientific evidence into recommendations for eating patterns to promote health and help prevent chronic diseases, many of which are linked to poor dietary quality, among the American population. Recommendations in the 2015 DGA focus on increasing dietary variety and nutrient-density and shifting to healthier foods, beverages, and eating patterns. However, the typical American diet does not align with these guidelines.

The primary aims of this project were to identify strategies to improve the variety and nutrient-density of the American diet and conduct clinical and epidemiological studies to assess their potential impacts on health. Secondary aims were to review topics that warrant attention in the DGA, compare U.S. guidance with recommendations of other countries, and evaluate components of dietary guidance that merit reconsideration.

Substituting mushrooms for meat at some meals would increase Americans' intake of vegetables, fiber, and non-animal protein and help align U.S. diets with DGA recommendations. A clinical intervention study compared the impact on satiety and gut health markers of adding mushrooms or meat to a typical American consumers' diet. In a randomized open-label crossover study, participants (n=32) consumed protein-matched amounts of mushrooms or meat twice daily for ten days, including at an in-person visit. During the last five days of each diet, participants completed a full fecal sample collection. Mushroom consumption impacted subjective satiety markers but not energy intake and led to few significant differences in gut health markers compared to meat consumption. After a mushroom meal, participants reported less hunger ($p = 0.045$),

greater fullness (p= 0.05), and decreased prospective consumption (p= 0.03) than after the meat meal. There were no statistically significant differences in participant ratings of satisfaction (p= 0.10) or in energy intake at an ad libitum meal. There were also no differences in breath hydrogen and breath methane measurements or with stool frequency, consistency, pH, or short chain fatty acid concentrations between the two diets. Mushroom treatment led to greater overall gastrointestinal symptoms, including gas and flatulence, than the meat diet on days 1 and 2 as well as higher average stool weight (p= 0.002). The higher stool weight and presence of undigested mushrooms in stool suggest that mushroom consumption may impact laxation.

Adults and children in the U.S. commonly consume "snacks," or energy outside of the traditional mealtimes of breakfast, lunch, and dinner. Replacing foods currently selected as snacks with nutrient-dense alternatives could lower the risk of nutrient deficiencies and excess nutrient consumption and improve the quality of the U.S. diet. Yet, while the DGA recommend selecting nutrient-dense foods, they do not provide a metric for evaluating nutrient-density. The Nutrient-Rich Foods (NRF) Index, a nutrient profiling method with scores that positively correlate with the Healthy Eating Index, was used to quantify the nutrient-density of foods frequently selected as snacks. Epidemiological datasets, including the National Health and Nutrition Examination Survey (NHANES) and the School Nutrition Dietary Assessment Study, as well as market research data were used to identify common snacks. Several common snacks, including yogurts, milk, fruit, nuts and seeds, and potato chips had relatively high NRF index scores, indicating nutrient density. Other frequently selected snacks including soft

drinks, pies and cakes, ice cream, and cookies had negative NRF scores indicating low nutrient-density. Nutrient-density scores may not provide new information about snacks at either end of a "nutrient-density spectrum," such as yogurt, fruit, soft drinks, and ice cream. If added to food labels, nutrient-density scores could serve as helpful tools for consumers to identify more nutrient-dense options among the foods located between the extremes.

Snacks as an eating occasion also merit attention in dietary guidance. The label ascribed to an eating occasion (i.e. "snack" or "meal") influences other food choices an individual may make on the same day as well as satiety after consumption. However, the DGA as well as the dietary guidance of several other countries, including Brazil, Canada, Japan, and Oman, do not directly address the healthfulness of additional eating occasions and also vacillate between defining "snacks" as an eating occasion and as a type of food ("snack food"). Dietary guidance could reimage "snack foods" to prevent "snack time" from becoming an occasion for overconsuming nutrient-poor foods.

Another component of dietary guidance that warrants reconsideration is the labeling of food groups on USDA's 2010 MyPlate guide, a visual food guide for educating consumers about dietary guidance. When the previous food guide, MyPyramid (2005), was replaced with MyPlate, the name of the "meat & beans" group was changed to the "protein group." The exclusion of dairy foods from the "protein foods" group of MyPlate illustrates the shortcomings of the new name. Previous research also shows that that consumers understand food-based terms better than nutrient-based terms. Changing the name of this group back to "meat & beans" group would provide important

clarification for consumers and educators regarding the content and dietary role of this group.

The DGA incorporate recent scientific evidence into recommendations for the U.S. population, however, these recommendations require more effective translation to the American public to impact public health.

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Section 1: Literature Reviews

Chapter 1: Mushrooms

Summary

Although mushrooms are a commonly consumed food, few nutrition studies have been published on them. The nutrient composition of mushrooms, which includes a combination of fiber and protein, suggests that eating mushrooms may influence satiety. The fiber profile of mushrooms, even white button mushrooms, includes known prebiotics, suggesting that mushroom consumption may impact gut health as well. However, little research has been conducted on the impact of mushroom consumption on satiety in human subjects, and no research has been published on the impact of mushroom consumption on human gut health. The increasing prevalence of chronic disease states, including obesity, in the U.S. makes understanding dietary influences on satiety and gut health timely and important. Given that long-term weight gain has been linked with a decreased consumption of whole foods like vegetables, nuts, fruits, and whole grains, focusing nutrition research efforts on whole foods, especially environmentally sustainable and safe foods like mushrooms, is vital to the improvement of public health.

Introduction

Mushrooms are a commonly consumed food,[1] but little nutrition research has been published on them. A recent review even listed mushrooms as a “forgotten” white vegetable, along with potatoes, turnips, and onions,[2] even though mushrooms are fungi,

not vegetables or even plants.[3] Yet, mushrooms are considered a vegetable (part of the "other vegetable" category) in U.S. dietary guidance materials.[3] Compared to other foods listed as "other vegetables" by the USDA's MyPlate food guide, mushrooms offer moderate quality protein[4] and a unique carbohydrate profile. Although the nutrient content of mushrooms varies based on growth medium, mushroom strain, environmental conditions, and preparation or processing methods, scientific literature generally indicates that mushrooms contain all nine essential amino acids (Table 1-1)[4] as well as several types of non-digestible carbohydrates including chitin, β-glucans, and resistant starch (Tables 1-2 and 1-3).[5–7]

While hundreds of different mushroom varieties are eaten around the world, the most commonly consumed mushroom is *Agaricus bisporus*.[8] *A. bisporus* describes three different types of mushrooms- white button mushrooms, crimini/brown cap mushrooms, and portabella mushrooms. The only differences between these three types of mushrooms are color and age. White buttons are white immature *A. bisporus*, crimini/brown cap mushrooms are brown immature *A. bisporus*, and portabellas are mature *A. bisporus*. This review will focus on white immature *A. bisporus* mushrooms, and from this point forward, the word "mushrooms" will refer to this type of *A. bisporus* fungi.

The nutrient profile of mushrooms[5,7] suggests that they may contribute to satiety, impact the diversity of the gut microbiota, and affect overall gut health. Mushrooms are less calorically dense and more fiber-rich than meat, which may help prevent weight gain when substituted for meat while still providing higher quality protein than some other plant sources.[9] The carbohydrate profile of mushrooms, which includes some known

prebiotics,[10] also suggests that they may impact gut health. However, there are limited data on the effects of mushroom consumption on satiety and even less data on the impact of mushroom consumption on the gut microbiota. None of the studies published so far on mushrooms and gut health has been conducted in a human population.

Satiety

With rising rates of obesity in the U.S., increasing scientific understanding of satiety is a topic of great importance for both clinical treatment and public health efforts. Research has long focused on satiety, a "strong factor in controlling food intake,"[11] as a contributor to weight status and weight maintenance. Satiety, the postprandial state in which hunger and further eating are inhibited, is responsible for the timing and intake of the next meal.[12,13] Satiating meals could result in decreased daily caloric intake and, over time, assist with weight loss and weight management.

Because both fiber and protein contribute to satiety, the combination of these nutrients in mushrooms suggests that mushroom consumption may influence satiety. While one serving of mushrooms contains only small amounts of protein and fiber (Table 1-4),[6,14] due to the small amount of calories in a serving of mushrooms, these two nutrients account for nearly 60% of the total calories in a serving of mushrooms.*

While many factors influence satiety, some of the identified physiological factors responsible for satiety include gut hormone secretion, gastric emptying, and gastric distension.[11] When chemoreceptors in the small intestine detect the presence of food,

* Assuming mushroom fiber provides 2 kcal/g

enteroendocrine cells release hormones, including the satiety peptides cholecystokinin, peptide YY, and glucagon-like peptide-1.[15] These peptides circulate in the plasma and "modulate short-term control of food intake."[16] Finally, the presence of nutrients along the gastrointestinal tract initiates a cascade of effects slowing gastric emptying and triggering the ileal brake mechanism.[17] Satiety is inherently acute as these effects after one meal typically last only until the subsequent meal. Yet, there is potential for satiety effects to be longer-lasting due to braking mechanisms along the gastrointestinal tract that further slow transit time, increase colonic fermentation, and delay desire for the next meal. Several of the mechanisms associated with satiety have yet to be defined.

Satiety can be difficult to assess. Historically, satiety assessments have relied largely on subjective ratings of fullness. Visual analogue scales (VAS) are commonly employed in satiety studies for subjects to rate perceived fullness after consuming a test food or beverage.[16,18–20] A more quantitative assessment of satiety involves measuring the energy intake of subjects provided *ad libitum* food following consumption of a test food or beverage.[16,18–20] In 1995, a research team generated a "satiety index" of common foods based on VAS ratings from subjects who consumed 240 kcal portions of different "test" foods and rated their hunger in 15 minute increments after consumption. Two hours after consumption, Holt et al. assessed the energy intake of these subjects at an ad libitum meal.[18] Foods rich in fiber and/or protein were associated with sustained "hunger control."[18] Subsequent studies and reviews have echoed those findings.[21–23]

While white button mushrooms contain a relatively small amount of satiating protein (3.09 g/100 g),[6] mushrooms protein is also of moderate quality[4,5] and has a

protein quality rating (protein digestibility corrected amino acid score, PDCAAS) of 0.66.[9] PDCAAS scoring evaluates protein quality based on limiting amino acids, fecal digestibility, and the protein needs of preschool-aged children, with higher values given to higher quality proteins.[24] The highest quality protein sources in this index are animal sources, such as milk and eggs (PDCAAS value of 1.00), while wheat protein has a PDCAAS value of 0.42.[24] Mushrooms have a higher protein quality rating than several other non-animal sources.[25]

In addition to their protein content, mushrooms contain fiber, which also contributes to satiety and feelings of fullness.[26,27] Foods higher in fiber, especially whole foods with naturally occurring fibers, take longer to chew and allow "more time for satiety signals," promote fullness, slow gastric emptying, and tend to have lower energy density.[13,23] White button mushrooms contain 28.5% (of dry matter, raw) to 38% (of dry matter, boiled) total dietary fiber.[5] Fiber can enhance satiety via multiple mechanisms including increased viscosity and bulking, resulting in decreased transit time and fermentation products, which generate hormonal feedback signaling satiety in the brain. An inverse relationship has been found between body weight and intake of high fiber foods.[13] As most Americans do not meet the recommended daily intake for dietary fiber,[28,29] low fiber intake may play a role in obesity rates.

Finally, the combination of protein and fiber could provide a dual mechanistic action that may have greater satiety impact than either nutrient on its own. The concept of combining two satiating macronutrients would suggest an additive or even synergistic effect as each macronutrient and food form exerts satiety effects by independent

mechanisms. While a limited number of studies have tested this combination,[20,30] even fewer have tested this concept with a whole food diet over more than week.

Relatively few studies exist on the satiating properties of mushrooms, but the studies that have been published suggest that substituting mushrooms for meat may lower caloric intake and contribute to weight loss. Cheskin et al.[31] compared mushroom and meat lunches for satiety response in individuals (n=76) with BMIs between 18 and 45 kg/m^2. There were no significant differences in ratings of hunger, satiety, or palatability by participants between the mushroom lunch weeks and the beef lunch weeks. However, average daily caloric and fat intake were lower ($p<0.0001$) during the two weeks of mushroom lunches compare to the two weeks of beef lunches. Mushrooms were also compared to meat in a parallel group yearlong trial in which obese adults (n=36) were asked to either substitute mushrooms for red meat at three meals per week or eat red meat at three meals per week (n=37).[32] Both groups were prescribed a 500 kcal energy-deficient diet for the first six months of the study. While both groups showed a lower BMI and waist circumference during the 6-month intervention, the mushroom group showed trends toward greater losses in weight, waist circumference, and BMI.

Gut Health

Emerging research suggests that, like satiety, the microbiota may also influence weight status.[33,34] The carbohydrate profile of mushrooms suggests that they may impact the gut microbiota and overall gut health as well. While a 2009 review suggested that

mushroom consumption may benefit gut health,[10] no human studies on the impact of mushroom consumption on the gut microbiota have been published.

While mushrooms contain very small amounts of carbohydrates known to impact gut health (Tables 1-2 and 1-3),[35] these components, in addition to the rest of the non-digestible carbohydrates in mushrooms, may promote gut health by increasing fecal bulk and generating short chain fatty acids. They may also promote the growth of beneficial bacterial in the colon.[35] Resistant starch, beta-glucans, and mannitol are known prebiotics, or "substrates selectively utilized by host microorganisms [that] confer a health benefit,"[36] within the gut.

Only studies in animals have been published on the effect of mushroom consumption on the gut microbiota. Yet results from these animal studies suggest a potential of mushrooms to function as prebiotics in the human gut. Adding 1% white button mushrooms to the purified diet of mice resulted in increased gut bacterial diversity, including increases in the Bacteroidetes phyla and decreases in the Firmicutes phyla compared with control-fed mice.[37] A second study[38] assessed the effects of a water extract of reishi mushrooms (*Ganoderma lucidum*) on the mouse gut microbiota. Chang et al. fed 2, 4, and 8% (w/v) *Ganoderma lucidum* solutions to mice fed a high fat diet via intragastric gavage for two months, which decreased the *Firmicutes: Bacteroidetes* ratios and body weight of obese mice. Additionally, while less relevant to human populations, an animal study in turkeys found that ground *A. bisporus* mushrooms added at 0, 10 or 20 g/kg feed for 70days increased cecal lactobacilli and *Bifidobacteria* counts in the 20 g/kg group ($P \leq 0.05$) compared to controls.[39] The lactobacilli counts were also

significantly higher ($P \leq 0.05$) in both groups (10 g/kg and 20 g/kg compared to the control group. A similar experiment conducted by the same research group, however, found that providing the same amount of dried mushrooms in the broiler chicken feed for 42 days slightly increased *Lactobacilli* and *Bifidobacteria* counts in the 20 g/kg group compared to controls and the 10g/kg group but had few other significant effects.[40]

Other markers of laxation and gut health beyond the microbiota such as stool weight, gastrointestinal short chain fatty acid production, stool pH, and stool consistency, have not been measured with mushroom feeding. Because mushrooms contain low digestible carbohydrates, beta-glucans, and other dietary fibers, they may affect these gut health markers as well.[10,41] Mushrooms may be able to positively impact the human gut microbiota and contribute to other changes beneficial for gut health.

Sustainability and Dietary Guidance

In addition to the potential of mushrooms to impact satiety and gut health, mushrooms are also an important food source to study because they are a source of inexpensive, easily cultivated protein. Mushrooms thrive on agricultural by-products, including livestock manure, recycled paper, straw, and coffee grounds, and require less space and time to grow and harvest than meat.[42] While mushrooms do not provide the same amount or quality of protein as meat, they have been used successfully in a sensory study to replace up to 80% of the meat in meat-based meals with mushrooms.[43] Substituting some of the meat, but not all of it, with mushrooms reduces calories, sodium,

and fat in meat-based meals, while actually increasing perceived flavor intensity, adding fiber, decreasing the perceived amount of sodium needed, and improving acceptance of vegetable protein by meat lovers.[43] The 2015 Dietary Guidelines for Americans encourage greater reliance on non-animal proteins and limiting consumption of meat, especially red meat.[44] However, few solutions beyond vegetarian substitutions and smaller portion sizes have been proffered as practical methods for decreasing meat consumption. As an accessible and palatable protein source, the cultivation of which has a low environmental impact, mushrooms merit additional research from food science, nutrition, and other food-focused fields.

Safety

As with many common agricultural products like potatoes, which are widely consumed and rich in nutrients but also produce the toxic compound solanine,[45] white button mushrooms contain nutrients (protein, vitamin D, and potassium) as well as agaritine, a potential carcinogen. Studies in mice and rat models throughout the 1980s and 1990s assessed the carcinogenicity of agaritine, finding that it exerted a weak genotoxic effect.[46–49] However, in 1987, agaritine was labeled a Group 3 carcinogen by the International Agency for Research on Cancer, indicating that it is "not classifiable as to its carcinogenicity to humans."[50] Both the U.S. Food and Drug Administration[45] and the Nordic Council of Ministers[51] also concluded that insufficient and inadequate evidence supports limits on mushroom consumption due to carcinogenicity concerns.

Conclusions

While more human intervention studies are needed, preliminary research on mushroom intake in humans and animals suggests that they may have beneficial effects on satiety and gut health. In addition, because of their protein content, classification in dietary guidance as a "vegetable," sustainability, and palatability, mushrooms are an important food source that merits further scientific investigation.

Table 1-1. Amino acid content of *A. bisporus* mushrooms

Amino Acid	**Amount in g per 100 g of *A. bisporus*[†]**
Leucine	7.5
Isoleucine	4.5
Valine	2.5
Tryptophan	2.0
Lysine	9.1
Threonine	5.5
Phenylalanine	4.2
Methionine	0.9
Histidine	2.7

[†] Adapted from: Miles PG, Chang S-T. M*ushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact.* CRC Press; 2004.

Table 1-2. Carbohydrate content of raw *A. bisporus* mushrooms

Carbohydrate	**Amount in g per 100 g of raw *A. bisporus***
Total Oligosaccharides	0.009‡
Galactooligosaccharides	0.009‡
Glucooligosaccharides	0.0‡
Fructooligosaccharides	0.0‡
Total Dietary Fiber	1.0§
Insoluble Dietary Fiber	1.3‡
Soluble Dietary Fiber	0.2‡
Chitin	0.60**
Beta-glucan	1.4**
	Percentage of *A. bisporus* Dry Matter
Resistant starch	12.3‡

‡ Dikeman CL, Bauer LL, Flickinger EA, Fahey GC. Effects of stage of maturity and cooking on the chemical composition of select mushroom varieties. *J Agric Food Chem*. 2005;53(4):1130-8. doi:10.1021/jf0485411.

§ USDA: Agricultural Research Service. USDA National Nutrient Database for Standard Reference. *Release 26*.:Nutrient Data Laboratory Home Page.

** Manzi P, Aguzzi A, Pizzoferrato L. Nutritional value of mushrooms widely consumed in Italy. *Food Chem*. 2001;73(3):321-325. doi:10.1016/S0308-8146(00)00304-6.

Table 1-3. Carbohydrate content of roasted *A. bisporus* mushrooms*

Carbohydrate	**Percentage of roasted *A. bisporus***
Total Dietary Fiber	4.9%
Insoluble Dietary Fiber	3.5%
Soluble Dietary Fiber	1.4%
Beta-glucan	1.76%
Mannitol	2.96%
Resistant starch	<2%

*Amounts determined by Medallion Laboratories 1/4/17 using AOAC 2011.25 for fiber determination, AOAC: 2022.02 for resistant starch, and an internal method for sugar alcohols determination

Table 1-4. Nutrient content of *A. bisporus* mushrooms

Nutrients per 85 g*	***A. bisporus* (raw)**[††]
Calories (kcal)	19
Protein (g)	2.6
Fiber, total dietary (g)	0.8
Vitamin D (IU)	6
Calcium (mg)	3
Potassium (mg)	270

*85 g is the Reference Amount Customarily Consumed (RACC) for fresh or frozen vegetables without sauce (21 CFR 101.12).

[††] Values from USDA National Nutrient Database for Standard Reference. *Release 26*: Nutrient Data Laboratory Home Page.

Chapter 2: Snacks

What Is a Snack, Why Do We Snack, and How Can We Choose Better Snacks? A Review of the Definitions of Snacking, Motivations to Snack, Contributions to Dietary Intake, and Recommendations for Improvement

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Summary

Around the world, adults are consuming energy outside of traditional meals such as breakfast, lunch, and dinner. However, because there is no consistent definition of a "snack," it is unclear whether these extra eating occasions are additional meals or snacks. The manner in which an eating occasion is labeled (e.g. as a meal or snack) may influence other food choices an individual makes on the same day and satiety after consumption. Therefore, a clear distinction between "meals" and "snacks" is important. The aim of this paper is to assess the definition of extra eating occasions, to understand why eating is initiated at these occasions, and to determine what food choices are common at these eating occasions to identify areas for dietary intervention and improvement.

Part I of this review discusses how snacking is defined and the social, environmental, and individual influences on the desire to snack and choice of snack. This section concludes with a brief discussion of the associations of snacking with cardiometabolic health markers, especially lipid profiles and weight. Part II addresses popular snack choices, overall snacking frequencies, and the demographics of frequent snackers in several different countries. This review concludes with a recommendation for nutrition policymakers to encourage specific health-promoting snacks that address nutrient insufficiencies and excesses.

Introduction

Because of the difficulty involved in defining "snacks" and "snacking," there is discrepancy in the literature about whether snacking prevalence has increased or remained static and whether snacking contributes to energy imbalance and weight gain[52,53] or facilitates weight maintenance and a lower body mass index (BMI).[54–56] Yet, we know that individuals are consuming energy outside of meals,[53,57–72] regardless of the overall prevalence of snacking or its impact on health. This review discusses the definitions and presentation of snacking in the current literature and snacking patterns in several areas of the world. Part I of this paper discusses how snacking is defined and the social, environmental, and individual influences on the desire to snack and choice of snack. This section concludes with a brief discussion of the associations of snacking with cardiometabolic health markers, including plasma lipid levels and BMI. Based on information from part I as well as cross-sectional data and government dietary guidelines,

part II of this paper evaluates popular snack choices in several different countries and how the nutrition science community can promote nutrient-dense snack options and choices. Given that snacking is still an eating occasion during which people consume energy and nutrients,[53,57–72] even if the impact of frequent eating on health remains largely unknown, choosing healthful snacks could help mitigate potential negative effects of snacking and contribute to promoting and facilitating nutrient-dense and health-promoting diets.[73]

Although several different definitions have been proposed in the literature, in this review, "snacks" will refer to eating foods or consuming caloric beverages between regular meals.[58,62,65,68,72,74,75] "Snack foods" will designate energy dense, nutrient-poor foods high in sodium, sugar, and/or fat such as cookies, cakes, sugar sweetened beverages, and chips.[63,64,70,72,76,77] "Snacking" refers to the act of eating a snack, regardless of whether healthful choices or "snack foods" are consumed.[53,60,64,67,69,70,72,77–79]

Part I: Definitions of Snacks, Influences on Snacking, and the Effect of Snacking on Metabolic and Cardiovascular Health

What is a "snack"?

How an eating occasion is labeled influences other food choices an individual makes on the same day and may even impact satiety after eating.[70,80–84] In addition, the delineation of different eating occasions impacts data collection about eating patterns and

their interpretation and is important for the research community to consider in order to collect accurate information.[84] Despite its potential to influence daily eating patterns of an individual as well as how data are collected and interpreted, the term "snack" does not have a static definition.[84] Several publications in the literature even comment on the definitional variation and difficulty of distinguishing meals from snacks.[74,77,84] Some current definitions of "snack" in the literature are based on the time of day of an eating occasion,[56,62,64,65,67,68,71] type of food consumed,[63] amount of food consumed, location of food consumption, or a combination of several of these factors.[68,72,74,85] Furthermore, some studies rely upon study participants to label their eating occasions, sometimes with[52,53,56–61,65,66,68–70,75,78,83,86] and sometimes without[63] providing them with a list of examples or controlled, defined labels. Provided labels, however, still vary by study. Several studies allow participants to categorize eating occasions as either meals or snacks,[52,56,57,66,70] while others separate specific meals (i.e. breakfast, brunch, lunch, dinner, small meal, main meal) from snacks.[53,59,61,68,69,83] Some studies further differentiate between snacks based on time of day (i.e. morning, afternoon, or evening snacks).[58,65,75,78]

In other studies, research teams or interviewers classify eating occasions for participants following reporting of food intake.[56,64,67,71,72,74,85] Some studies that use cross-sectional data, especially from multiple studies, even re-classify participant-defined eating occasions.[52,53,56,60,62,69,75,86] To re-classify, some research teams collapsed two eating occasions, such as breakfast and brunch, into a single occasion[83] or combined all eating occasions within 15[52,53,56,60,69,86] or 30[71] minutes of each other into one eating

occasion. However, some studies did not specify how food intake data was separated by eating occasion.[62]

Having participants define eating occasions without any designated parameters could introduce considerable variety into snacking data. Although this phenomenon has not been studied extensively, a few surveys have specifically assessed interindividual variations in the definition of "snacks."[87–89] Two surveys were conducted on American college campuses.[87,88] Undergraduate students associated snacks with the following cues: eating alone, short eating periods (10 minutes), disposable utensils, lower food and nutrition quality, and most significantly, standing while eating.[87] Generally, respondents perceived snacks to be small portions of packaged, inexpensive, and nutrient-poor foods and defined "snacks" as a specific set of foods.[87] The second survey of college students found that the time of day and location of consumption also factored into whether an eating occasion was considered a meal or snack.[88] A third survey conducted in England found that respondents (n=121) defined snacks, snacking, and snack foods differently.[89] University students and staff were mailed surveys and asked to define one term (snack, snacking, or snack foods) and describe (including food, location, company, time of day) the last time they had eaten a snack, snacked, or eaten snack foods.[89] All questions were open-ended. This survey was followed with a second survey (n=86) on the other two terms. Although the differences between the definitions of "snacks," "snacking," and "snack foods" were not significant, the data did suggest "conceptually consistent differences in usage" among the terms based on location of food consumption, food choice, and time of day.[89] For instance, "snack food" was more likely to be eaten later in

the day (after 6:00pm) whereas a "snack" was more likely to be consumed early in the day.[89] However, in the conclusion of this study, the authors expressed hesitance about defining any of these terms and suggested that due to the considerable variation among participant responses, the root word "snack" should be avoided in research questionnaires.[89] The participants in all three of these surveys are part of very specific populations and, therefore, the results cannot be widely extrapolated. Nonetheless, the variation in the definition of "snack" from this very limited audience alone suggests the possibility of even greater disparity in "snack" definitions in populations of greater age, ethnic, and cultural diversity.

As one example, data from the National Health and Nutrition Examination Survey (NHANES) in the U.S., which relies upon participant definitions of eating occasions, is intended to represent the entire American population.[90] NHANES data do show some differences in snack definition, most notably that some respondents "defined foods eaten at the same time as both a snack and a meal."[53,60,69] These respondents may define snacks by type of food consumed (i.e. snack foods) rather than by the time of day consumed. Although researchers can recode eating episodes by time of consumption, the presence of these single eating occasions with multiple codes suggests that participants of NHANES define snacks differently. Researchers cannot feasibly account for all of the variations.

Although the semantics of eating occasion labels may seem trivial, an individual's definition of an eating occasion as a snack or as a meal may influence their food selection.[79,81,83,84,87] An analysis of NHANES data from 1988 to 1994 found that individuals who reported skipping a meal but eating several snacks had less healthful

overall nutrient intakes compared to individuals who ate three meals, with or without snacks.[83] Diet quality was assessed by macro- and micronutrient intake, including cholesterol, Vitamins B_6 and C, folic acid, calcium, magnesium, iron, sodium, potassium, and fiber.[83] Individuals who ate all three meals as well as snacks had the "highest intakes of all micronutrients examined, except cholesterol, Vitamin B_6, and sodium."[83] People who skipped breakfast but ate two snacks had the "lowest intake of all micronutrients except sodium."[83] In this study, individuals who classified their eating occasions as meals, therefore, seemed to choose more nutrient-dense foods.

In addition to affecting micronutrient intake, defining eating occasions as meals versus snacks influences food choices later in the same day.[81] An intervention study with 138 undergraduate students demonstrated that individuals report feeling less satiated by a "snack" than a "meal," even when the two eating occasions are isocaloric.[81] Individuals also tend to consume more calories at an eating occasion following a "snack" than following a "meal."[81] Previous food diary[70] and intervention[79,82,91] studies have also indicated that eating between meals does not affect the amount of calories eaten at the next meal. The results of these studies[70,81–83] suggest that simply the way an eating occasion is labeled may influence choice of food, satiety, and daily caloric intake.

To clarify data in the literature, especially data from large epidemiological studies, it may be prudent to avoid the word "snack" on forms and interview questions to minimize confusion about what it means to eat a "snack."[89] Instead, participants could be asked to simply record meals and food or caloric beverage items consumed between meals.

Defining Snacks: Nutrition Policy

Government-issued dietary guidelines could also benefit from the use of a clear and consistent definition of snack and snack food or the elimination of these terms altogether. Based on the literature discussed in this section, consumers seem to define "snacks" and "snack foods" differently.[87–89] Yet, neither of these terms have a clear definition in some government-issued dietary guidelines.[92–97] The 2010 Dietary Guidelines for Americans (2010 DGA), for instance, encourage decreasing consumption of "snack foods" but also provide few suggestions for "snacks." While their intended meaning may not seem difficult to discern, the plethora of "snack" definitions among the American public may complicate the interpretation of these guidelines by some sectors of the general public, the intended audience for these guidelines.[93] In order to clarify recommendations, dietary guidelines need to provide definitions of "snack" and "snack foods," especially since the label applied to an eating occasion can influence nutrient intake, satiety, and food quality.[70,79–84,87]

Motivations to Snack

Similar to the definitions of snacking, the desire to snack depends on several different factors. The motivations to snack discussed in this review include hunger,[77,79,98] location,[88,99–101] social/food culture and environment,[77,102–108] cognitive factors,[109–113] and hedonic eating.[114–117]

Hunger

While snacking when hungry tends to be associated with the consumption of health-promoting foods, snacking in the absence of hunger leads to the consumption of fat, sugar, and sodium-rich foods.[77] Unnecessary snacking promotes "weight gain and poor nutrition,"[77] and the results of several studies by Chapelot et al. support this hypothesis.[79,98] In one study, habitual nonsnackers were offered a snack between lunch and dinner.[98] Although all of the participants consumed at least one food item offered as a snack, the researchers found no evidence of a biological cue (hunger score change, decrease in insulin or glucose levels) prompting a desire to eat.[79] Chapelot et al. concluded that these participants ate because food was available even in the absence of biological cues, an example of unnecessary snacking. However, the control group of regular snackers did adjust the timing and size of their next meal after consuming a snack.[79] According to these studies, non-habitual snackers lack a biological motivation to eat snacks and, for these "nonsnackers," snacking without hunger leads to increased energy consumption, which can cause eventual weight gain.

Location

Location may impact food selection for snacks[88,100,101] as well as portion size.[99] While eating at home or at work is associated with more healthful food choices for snacks, eating at other locations is associated with larger snack sizes[99] and higher fat and

lower fiber content.[100,101] One survey asked British and Australian college students to "construct a 'typical' lunch, dinner, or snack for 11 specific locations" using a list of 51 foods.[88] Locations ranged from eating while watching television at home to eating in an airplane or on a camping trip.[88] This study used cluster analysis to group foods by location and eating occasion and found that, though participants grouped some foods by eating occasion, the foods selected as appropriate for each eating occasion more often depended on the location of food consumption.[88]

The results of three cross-sectional studies also show an impact of eating location on food choice. A recent cross-sectional study of snacking habits of Norwegian adults (n=1787) found that snacks eaten in the workplace had the most favorable nutrient profile and generally consisted of less energy and added sugars but more protein than snacks consumed at home, at restaurants, or traveling.[100] Another cross-sectional study of Irish adults (n= 958) assessed the nutrient contributions of foods in their daily diets by location of consumption.[101] This study did not distinguish between eating occasions, but the results show that foods eaten outside the home generally had more fat, less fiber, and fewer micronutrients than foods eaten at home.[101] Finally, a study of 115 Northern Irish children ages 5 y to 8 y found that the snacks children ate outside the home tended to be significantly larger than snacks eaten in the home.[99] However, for this group, the foods selected for snacks were similar both in and outside the home, which may be due to parents bringing snacks from the home for their children to eat outside the home.[99] Although not all of these studies look specifically at snacks, where individuals eat may

influence the nutrient profile and portion size of the foods they choose at different eating occasions, including snacks.[95–97]

Social and Food Culture and Environment

Snacking can also be influenced by social culture, food culture, and socioeconomic status.[77] While a comprehensive discussion of the plethora of environmental factors that influence eating is beyond the scope of this review, some factors relevant to snacking, including social modeling and food insecurity, will be addressed.

Several studies have shown that the amount of food consumed by eating companions impacts portion size, an effect referred to as "social modeling."[102,103] According to a recent review,[118] this effect has primarily been studied in the context of snack food consumption. If an eating companion eats a large portion of food, the person eating with them also tends to eat more. The converse is true with small amounts of food. Even if an eating companion is not present, environmental cues about earlier individuals' food intake and choices, such as empty food wrappers, can influence intake.[102,119] The enhanced influence of eating companions during snack times may be due to the lack of an "eating routine" or "script" for snacking as an eating occasion[103,104] while meals tend to be more constrained by certain behaviors or places.

Snack consumption may also be initiated because of celebratory social occasions as well as the availability of or desire for tempting food. One research team developed a "Reasons to Snack" inventory with 35 different options and used this inventory in a study

of 1,544 adults.[105] This inventory was specifically developed to assess individuals' reasons to consume unhealthy snacks containing large amounts of fat or sugar, and this study found that the most common reasons for consuming unhealthy snacks included celebrating at a party or special occasion or craving a tasty food.[105] Another study asked 55 adults to keep a diet diary for five days and rate their reasons for eating using a similar scale with only 13 items.[106] In this study, the most common reason for consuming unhealthy snacks was that "they looked or smelled so tempting" (55% of snacking occasions), followed by "hunger" (49%) and "needing energy" (23%).[106]

However, in some countries, including France,[70,79] the Philippines,[75] and Mexico,[65,75,120] a fourth "meal" or snack is part of a traditional meal pattern. The French have "goûter" between lunch and dinner.[77] A small meal between lunch and dinner, *merienda*, is customary in the Philippines.[75] In Mexico, a mid-morning meal (*almuerzo*) is relatively common.[65,120] In these countries, therefore, tradition may motivate snacking,

In food insecure populations, however, snacking may be adopted as a strategy to skip meals. [107] Food insecure individuals have limited or uncertain "access at all times to enough food for an active, healthful life."[107,108] NHANES collects food security data on individuals through a Food Security Survey Module (FSSM).[107,108,121] Recently published studies on snacking and food security[107,108] use information from the 1999-2002 FSSM, which divides individuals into four groups: food secure, marginally food secure, food secure without hunger, and food secure with hunger.[108] These studies found that individuals who are food insecure without hunger snack more often, eat larger meals, and may consume more calories from snacks than food secure individuals.[107] Food insecure

women without hunger consumed more energy at snacks, and food insecure men without hunger consumed more energy at meals than their food secure counterparts.[107] Because the major energy source for snacks among food insecure adults was "sugar, sweets, and beverages," this trend towards increased snacking indicates that snacks may serve different roles in the diet and have different health effects based on socioeconomic status.[107,108]

Distracted Eating

Other motivations to consume snacks include distracted eating and the association of eating with certain activities. Several articles have been published on how eating while distracted affects the amount of food individuals choose to consume later in the day.[109–111] For instance, eating lunch while watching television (TV) or playing video games tends to increase the amount of snacks people eat later.[109–111] However, as stated in a recent meta-analysis and systematic review, this finding has been replicated primarily in relatively homogenous populations with healthy BMIs and an age range of 20 y to 47 y.[109]

In addition to affecting later memory of food consumed, watching television has also been associated with the number of snacks consumed per day.[112] In Canadian college students (n=613), "medium" to "high" viewers of TV (where "high" was ≥4 hours of TV daily and "medium" was between 1 and 4 hours of TV) snacked more frequently than "low" TV viewers.[112] Snack frequency was assessed using a five-point Likert scale that asked participants to rate on how often they snacked while watching TV ("never" to

"every day").[112] The results of this study suggest that individuals who watch TV frequently perceive themselves as snacking more frequently while watching TV.

The reasons for greater snack consumption while watching TV have not been fully explained in the literature, but one research team assessed the impact of different types of television programs ("boring" versus "engaging") on food intake in normal weight college-aged female subjects (n=18).[113] After a four hour fast, participants had free access to both chocolate candies and grapes while either watching TV or reading for 30 minutes. A "non-engaging" text for reading was used as a control. Participants consumed significantly more snacks (by mass) during both the boring TV condition (P=0.009) and the text condition (P=0.05) relative to the engaging TV condition. However, participants ate significantly more grapes than chocolate candies in all conditions (P=0.006). Although the study population was limited to young women and most of the snacks eaten were fruit, boredom did seem to contribute to the decision to snack.

These studies suggest that eating while distracted may contribute to reduced satiety and increase consumption at the next eating occasion. "Boring" distractions may increase snack intake even more. If individuals are "multi-tasking" while snacking, they may eat more of a snack or consume more food at their next meal. More research is needed in this area with more diverse study populations over longer time periods to determine how distracted eating affects intake and body weight.

Hedonic Eating

In addition to being motivated by distraction, snacking may also be motivated by the rewarding properties of food, or "hedonic eating." One personality model, reinforcement sensitivity theory, asserts that regulation of food intake may be driven by an individual's sensitivity to reward.[114] The initial study assessing connections between reward sensitivity and eating behaviors surveyed female college students (n=99) with questionnaires about food cravings and their sensitivity to punishment and reward.[114] Women more sensitive to reward had higher BMIs and higher food craving scores ($P<0.05$).[114] Similarly, a cross-sectional study of 1,104 adolescents found that 14- to 16-year-olds "sensitive to reward" consumed more energy-dense snacks and sugar-sweetened beverages than individuals less "sensitive to reward."[115]

Yet, in another study, initiation of eating in the absence of hunger was not significantly correlated with sensitivity to reward.[116] This study provided 50 adults with a "snack taste test" of chocolate candies, which participants were instructed to consume until satiation. Immediately following the first test, participants were given a second, unanticipated, and voluntary opportunity to consume a different kind of chocolate candy. Not all adults accepted the second taste test. However, the only significant difference between the adults who chose to participate in the second taste test and those who did not was that the adults who participated had significantly higher inhibitory control scores than the adults who declined ($P=0.03$). There were no significant differences in BMI, impulsivity, hunger, or food reward sensitivity between the two groups.

Therefore, while two cross-sectional studies[114,115] show significant associations between reward sensitivity, BMI, food cravings, and snack food consumption, the results of an intervention study did not support these findings. This difference may be due to the different populations assessed in each study. However, this area of study is relatively new, and the connections between sensitivity to reward and eating habits, especially snacking habits, merit further investigation.

Snacking, Heart Health, and Weight

Whether snacking is initiated because of hunger, regular eating patterns, or other psychological or physiological cues may be predicative of its effect on weight.[56,77,79,98,114] The health impact of eating frequency may depend on how an individual defines an eating occasion (a snack versus a meal) as well as their motivation to eat, food choice, age, gender, and socioeconomic group.[62,65,69,71,72,79]

Heart Health

The only consistent link between snacking and a health outcome appears to be its association with improved cardiovascular health markers, including lipid profile (cholesterol and triglyceride levels) and blood pressure.[61,122,123] Frequent eating may improve lipid profiles and decrease the risk of cardiovascular disease.[61,123] A review article assessing the effects of feasting (1 large meal daily) versus "nibbling" (3, 6, 9, 12, or 17 small meals daily) found that the "nibbling" pattern was associated with lower total

cholesterol, LDL cholesterol levels, and blood pressure.[123] An additional study found that more frequent meal consumption (more than one to two meals per day) resulted in lower total and LDL cholesterol.[122] Although the results of this review and study assess a pattern of "frequent eating" rather than "snacking," they both suggest that consuming food more often throughout the day, an eating pattern that could be due to snacking, improves lipid levels and blood pressure.

Weight

As several recent reviews indicate, the effects of eating frequency on weight are not well understood.[55,77,79,124,125] "Snacking" specifically does not have any unambiguous correlations with weight and has been associated with healthy weight maintenance and weight gain as well as both high diet quality and low diet quality.[78,86] Reviews on the associations between snacking and weight in both adults[124] and children[126] report inverse correlations between snacking and adiposity. However, one of these reviews also notes that the correlation becomes positive when adjusted for underreporting.[124]

A recent cross-sectional study of adults (n=10,092) in England reports a helpful nuance to these different associations between weight and eating frequency.[76] In this study, snacking was inversely associated with body fat in normal weight individuals (BMI<25) but was positively associated with waist circumference and subcutaneous fat thickness in overweight and obese men and women.[76] Choice of snack also mitigated these associations. Overweight and obese participants tended to eat more snack foods like "crisps, chocolates, ice cream, and sweets" and less "yogurt and nuts" than the normal

weight participants.[76] Based on this study's results, pre-existing health status may influence snack choice and the effect of snacking on weight.

Part II: Current Snack Choices, Snacker Demographics, and Recommendations for Change

The remainder of this paper focuses on the foods and beverages that people choose to consume for snacks, the demographic profile of snackers, and suggestions for how the nutrition science community can recommend snack choices to better fulfill nutrient insufficiencies and avoid nutritional excesses. Information from several countries will be addressed but due to the authors' language proficiencies, data for Part II was limited to countries for which government-issued dietary guidelines were available in English or French and at least one study about snacking habits was available in English. Language presents a significant limitation to this worldview of snacking because it is not possible to know whether data on other countries is missing due to language barriers or a lack of data. In addition, the data discussed in this section is limited by the study populations assessed. Nationwide survey data about snacking patterns was not available for all countries and, therefore, some information used in this section relies on data from small, homogenous populations.

Foods for Snack

Food preferences for snacks are similar in several areas of the world. In the U.S., "salty snacks, desserts, candy, and sweetened beverages" are popular snack choices,[72]

and salty snacks have become especially popular since 1977.[69] In 2006, salty snacks including chips and nuts comprised 14.3% of total snacks consumed.[69] Salty snacks, including crackers, popcorn, and pretzels are also popular among Canadian youth.[67] From 1977 to 2006, the preference for sweet snacks in the U.S. decreased overall, but in 2006, desserts still comprised 19.6% of snacks.[69] Milk/dairy and fruits/juices have also become less popular snacks in the U.S. as well.[69] Yet, while fruit and sweets have declined slightly as snack selections in the U.S., they are very popular snacks in Mexico, Brazil, China, Oman, and France.[64,65,70,72,127] Fruit is the most common snack food in Mexico,[65] and one of the most popular snack items in Brazil.[64] Other popular snack items in Brazil are other sweets, desserts, and "salgados (fried/baked dough with meat/cheese/vegetable)."[64] Similarly, among Greek adults, two of the most popular snack items are desserts (chocolates, cakes, and ice cream) and savory pies.[57] In China, both fruits and grain-based foods are popular snacks.[72] Snacks in France also tend to include sugary grain-based foods, including "sweets, cereal bars, [and] biscuits,"[70] and Canadian children tend to also choose sweet grain-based products.[67] In Finland, however, the same foods are consumed at snacks and meals.[71] With the exception of fruit, many of these snacks fit the profile of "snack foods" and are relatively nutrient poor and energy-dense. Therefore, based on cross-sectional data, the choice of foods eaten for snacks is an area of concern for public health.

Beverages as Snacks

The increased consumption of caloric beverages as snacks also merits concern because sweetened beverages provide energy and few, if any, other nutrients. In the U.S., the energy density of beverages consumed as snacks has been rising since 1977.[69] From 1977 to 2006, the "percentage of snacks that consisted of beverages only" increased by 4%, and beverages now comprise about 100 kcal a day in the diets of American adults.[60,69] Beverages are also popular snacks among American children.[128] In Mexico, beverages (milk, soda, coffee, and tea) were among the top 5 snacks for all age groups.[65] Sweetened coffee and tea and sugar sweetened beverages were two of the top five snacks in Brazil,[64] and beverages overall are a popular snack category in China.[72] Coffee is one of the top three favorite snacks in Greece,[57] soda is a snack in France,[70] and tea is a popular snack among Omani girls.[127]

Snacking Demographics

"Snacker demographics" were evaluated for the following countries: Australia, Brazil, Canada, China, England, Finland, France, Greece, Mexico, Sweden, Switzerland, the United Arab Emirates, and the U.S., and snacking occasions were respondent-defined. Although a large proportion of adults and children in several of these countries snack, recommendations for snack choices could be further directed towards sectors of the population, such as women and young adults, who snack frequently in certain regions.

In some countries, including Brazil, Mexico, Canada, the U.S., Greece, and France, snacking contributes significantly to daily energy intake. In both Brazil and Mexico, national survey data show that about three-quarters of the population (74% in

Brazil, 73% in Mexico) consume an average of 1.6 snacks per day.[64,65] However, snacks contribute a more significant amount to daily energy intake of Brazilians (21% of daily intake) than to Mexicans (12% of daily intake).[64,65] "Heavy snackers" (three or more snacks per day) from Brazil receive about 35% of their daily energy intake from snacks.[64] In both Canada and the U.S., snacks comprise almost a quarter of the daily energy intake for adults: 23% of energy intake for Canadians and 24% for Americans.[68,69] In Greece, a small cross-sectional study (n=200) showed that snacks comprise 33.5% of daily energy intake, or 628 kcal, for adults.[57] Eighty-seven percent of the adults surveyed for the Greek study consumed snacks.[57] In a dietary intake study of 54 French adults, snacks provided an average of 18.5% of their daily energy intake, and these adults ate snacks on 20 of the 28 days they were asked to keep a food diary.[70]

In the countries for which snack data was available by gender and age, women tended to snack more often than men, but there were no discernable global trends by group. Small meals and snacks are common among women in Australia, China, Switzerland, Sweden, the United Arab Emirates, and the U.S.[58,60–62,69,72,129,130] More men are "snackers" in Finland, however,[71] and in Greece, the snacking habits of men and women are similar.[57] In terms of age, Brazilian adults over 60 y consume more energy from snacks than younger adults,[64] but Canadian adults over the age of 71 y consumed the lowest portion of their daily energy intake from snacks (16%).[68] Adult snackers in the U.S. and Mexico, by contrast, tend to be between the ages of 19 y and 39 y.[65,69] In Canada, adolescents ages 14 y to 18 y consume the most energy from snacks, with males in this range consuming about 30% of their daily calories from snacks and females

consuming about 28%.[68] Similarly, American children receive about 27% of their daily energy intake from snacks,[128] and in both China and Mexico, children snack more frequently than adults.[65,72] In the United Arab Emirates, adult women snack more than children, but data on Emirati men's snacking habits were not available for comparison.[62]

Snack Recommendations: Nutrition Policy and Nutrient Insufficiencies

Although the dietary guidelines of several countries mention snacks or snack foods (Table 2-1), some of them[92,94,95,97,131,132] caution against consuming sweet, savory, or salty snacks but provide few, if any, suggestions for health-promoting alternatives. For instance, the Nordic Nutrition Recommendations, the Omani Guide to Healthy Eating, and the Australian Eat for Health Guide recommend limiting "snack foods" as well as sugary and "savory snacks" because of their high salt, fat, and sugar content.[92,94,132] However, the Nordic Recommendations list no options for healthy snacks,[132] the Omani Guide recommends simply choosing snacks "wisely,"[92] and the Australian guidelines suggest only "legumes, nuts, and seeds" for snacks.[94] The snack suggestions in the Brazilian dietary guidelines similarly list few foods as appropriate "snacks"- milk, yogurt, and nuts.[131] The 2015 Scientific Report of the Dietary Guidelines Advisory Committee recommends selecting "healthy" and "smart snacks,"[97] but does not list "healthy" snacks or define "smart" snacks. In addition to clarifying a definition of snacks and snack foods, these dietary guidelines should offer suggestions of health-promoting snack options.

A few countries, Greenland, Sweden, France, and Switzerland,[96,133–136] already provide specific suggestions for snacks that include more options than dairy or nuts. In Greenland, snacks are recommended as an eating occasion, and snacking specifically on "a piece of fruit or a vegetable, crisp bread or dried fish" is recommended.[96] The Swedish Nutrition Recommendations suggest bread and margarine sandwiches, fruit, milk, and occasional sweets as snacks,[137] and Le Guide Alimentaire Pour Tous from France makes specific snack suggestions, including fruit, bread with butter and jam, and raw vegetables, for individuals who prefer to eat frequently.[134] With a two- page document on healthy snack choices for morning and afternoon snacks that includes fruits and vegetables listed by season as well as nuts and grain and dairy products, Switzerland provides the most comprehensive list of snack suggestions.[136] Although these guides also do not distinguish clearly between "snacks" and "snack foods," these guidelines could serve as models for other countries in developing snack recommendations.

The nutrient insufficiencies and excesses of certain countries could also be used to develop snack recommendations and even formulate specific snack foods. While few countries recommend specific food for snacks, countries with official dietary guidelines do tend to have population-level recommendations regarding the inclusion of certain nutrients or foods in the diet. Snack foods rich in important nutrients that rely on the preexisting snack preferences of different populations could contribute to facilitating nutrient-dense and health-promoting diets.

For example, the 2010 DGA identifies potassium, dietary fiber, calcium, and vitamin D as "nutrients of concern" because their intake is low enough to be of concern

for public health.[93] The DGA therefore recommends that Americans consume more vegetables, fruits, whole grains, milk and milk products, and seafood to address these insufficiencies.[93] These recommendations, in conjunction with current snack food preferences, could be used to develop recommendations for health-promoting snacks that are rich in the nutrients of concern.[73]

Nutrients of concern could also be used to guide the development of new snack foods. This strategy has been implemented in rural India, where one food company introduced beverages and snacks formulated to address specific nutrient needs, including water, iron, and folic acid, in 2011.[138] One of these snacks, made from extruded grains, contains 25 percent of the daily iron needs of adolescent girls as well as 50 percent of their recommended dietary allowances of thiamin, Vitamin B_{12}, and folic acid.[139] This snack is primarily intended to address the nutrient insufficiencies of adolescent females at risk of developing anemia due to low dietary iron intake,[138] and full nutrient data on this snack was not readily available to assess its overall nutrient profile. The development of health-promoting snacks could be an important area for collaboration between food companies and nutritionists, and this strategy of developing or recommending snacks that target specific insufficiencies and certain populations could be adopted in other countries for which similar data is available (Table 2-2).

Limitations

The lack of a consistent definition of "snack" in the literature impacted the collection and interpretation of information for this review. Research articles for this

review were identified via database searches using the term “snack” and from the bibliographies of relevant articles. However, snacking can also be discussed in articles about eating frequency, eating occasions, dietary habits, dietary patterns, frequent eating, and small meals. Evaluating information on all of these topics was beyond the scope of this review.

Furthermore, the information about snack preferences and demographics relies on limited amounts of data to draw conclusions about extremely large and diverse populations, and not all of this data is recent. Some of the data used for this review has not been updated for over ten years. Since other studies suggest that a shift in eating behaviors has occurred since that time,[69] some of this data is likely no longer accurate.

Conclusions

Snacks, snacking, and snack foods are difficult to define and study. The definition of and motivation to snack depend on external factors like time of day, type of food, food availability, and location, among others. Yet, the impact of frequent eating occasions on health outcomes, including weight gain, remains largely unknown. The literature suggests that consumption of nutrient-poor snacks may be associated with high BMI, eating in the absence of hunger, eating away from home or work, social modeling, and food insecurity. Even though these factors may be associated with poor dietary choices in some populations, the motivation to snack as well as the health impact of snacking are subject to considerable interindividual variation that merits further investigation. Because heavily salted, sweetened, and high-fat foods such as chips, desserts, and sugar-sweetened

beverages are still the most popular snacks in several different countries, dietary guidelines could reimage “snack foods” to prevent “snack time” from becoming an occasion for overeating nutrient-poor foods.

Table 2-1. Recommendations on snacks and snacking in the dietary guidelines of several countries and regions‡‡

Country	Snacking Recommendation
Australia	The Australian Dietary Guidelines relies on definition of snacks as a category of discretionary foods ("snack foods") to be consumed in limited amounts. The guidelines mention that "legumes, nuts, and seeds can be eaten as snacks."[94]
Brazil	Brazil's Dietary Guidelines discourage snacking between meals but suggests that individuals with higher energy needs consume small meals of fruit or "milk, yogurt, or nuts."[131]
Canada	Canada's Food Guide recommends limiting salty snacks and eating vegetables and fruit for snacks. For women of childbearing age, their guide encourages fruit and yogurt for snacks.[95]
England	England's National Health Service recommends snacking on dried fruit in the evening as a way to eat more fruit and also recommends nuts and fresh fruit as snacks. The NHS website has a page listing several low calorie snack suggestions.[133]
France	Le Guide Alimentaire Pour Tous recommends consuming a

‡‡ Data for this table include countries with dietary guidelines in English that contain a direct reference to snacking or best snacking choices.

	regular snack instead of eating mindlessly or snacking continuously. For snack foods, this guide recommends yogurt, milk, fruit, fruit juice, vegetables, or some bread with butter or jam.[134]
Greece	This guide recommends a few foods (nuts, seeds, fruit) as snacks as long as energy intake does not exceed energy expenditure.[135]
Greenland	Greenland's guide recommends limiting snack foods to one time per week but does recommend eating small healthful snacks such as "a piece of fruit or a vegetable, crisp bread or dried fish" between meals.[96]
Nordic Countries	The Nordic Nutrition Recommendations only discuss "snack foods" and recommend limiting them due to their high salt, fat, and sugar content.[132]
Oman	The Omani Guide to Healthy Eating suggests choosing snacks wisely and recommends choosing low-calorie and nutrient-dense foods. "Snack foods" are discussed as a major source of fats.[92]
Sweden	Swedish Nutrition Recommendations state that two or three snacks may be included each day as part of a healthful diet.[137]
Switzerland	Switzerland provides an entire page of healthy snack ideas, which includes fruits, vegetables, whole grain breads, cheese, yogurt, milk, and nuts but recommends against sweets and fatty, salty snacks.[136]

United States	The 2010 DGA recommend “raw, cut-up vegetables” and fruit as snacks.[93] The 2015 Scientific Report of the Dietary Guidelines Advisory Committee recommends decreasing snack food intake, as it is a high contributor to energy, sugar, and saturated fat intake and expresses concern over how snacks tend to be less nutrient dense than actual meals. It also suggests choosing “smart” snacks.[97]

Table 2-2. Nutrients of public health concern around the world

Country or Region	Nutrients of Public Health Concern	Source
Australia	Folic Acid, Iodine, Iron, Vitamin D	Australian Institute of Health and Welfare[140]
Brazil	Fiber, Vitamin A	FAO: Nutrition Country Profiles[141]
Canada	Calcium, Iron, Potassium, Vitamin D[142]	Health Canada[142]
China	Energy, Iodine, Iron, Vitamin A	FAO: Nutrition Country Profiles[143]
France	Calcium	Agence Française de Securité Sanitaire des Aliments[134]
Greece	Calcium, Folic Acid, Iodine, Iron	Ministry of Health and Welfare: Supreme Scientific Health Council[135]
Mexico	Iodine, Iron, Vitamin A	FAO: Nutrition Country Profiles[144]
Nordic Countries	Folic Acid, Iodine, Iron, Vitamin D	Nordic Council of Ministers[132]
Oman	Calcium, Fiber, Folic Acid, Iron, Vitamin D, Zinc	Department of Nutrition: Ministry of Health of Oman[92]

Persian Gulf Countries	Calcium, Iodine, Iron, Vitamin A, Vitamin D	Arab Center for Nutrition: Nutrition and Health Studies Unit of Bahrain[145,146]
Switzerland	Folic Acid, Iron, Vitamin D	Federal Office of Public Health[147]
United Kingdom	Folic Acid, Iron, Magnesium, Selenium, Vitamin C, Vitamin D, Zinc	Proprietary Association of Great Britain[148]
United States	Calcium, Fiber, Iron, Potassium, Vitamin D	Scientific Report of the 2015 Dietary Guidelines Advisory Committee[97]

Chapter 3: Dairy Foods

Dairy Foods: Current Evidence of their Effects on Bone, Cardiometabolic, Cognitive, and Digestive Health

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Summary

Dairy foods have long been considered nutrient-dense and health-promoting products that offer many health benefits to their consumers. This review is an overview of the health benefits associated with them, drawing from recent research conducted on the associations of dairy food components with bone, cardiometabolic, cognitive, and digestive health in cross-sectional and intervention studies. Each section details the associations of dairy with a certain aspect of health and focuses on the benefits milk product consumption may have on the prevention and management of chronic health conditions such as osteoporosis, the metabolic syndrome, and dementia. Dairy food components, as well as the potential biological mechanisms responsible for their effects on health, are also addressed. Although several of the biological mechanisms warrant further research, current evidence suggests that dairy consumption confers some beneficial effects to bone, cardiometabolic, cognitive, and digestive health. Due to its

nutrient profile and the current evidence of its benefits, at least 1 daily serving of a dairy item is recommended by the dietary guidelines of several countries. Yet, even in the United States, many individuals do not consume the recommended 3 cups of dairy foods a day. Therefore, this review concludes with a description of the current public health impact of dairy food research as well as recommendations for the food industry to formulate dairy foods that are both palatable and health-promoting for consumers.

Introduction

Chronic health conditions are the leading causes of death worldwide[149] and contribute significantly to healthcare costs and decreased quality of life for millions of people. The field of nutrition science has seen much time and resources spent on studying the prevention of chronic health conditions, including metabolic syndrome, osteoporosis, dementia, and digestive disorders. As older adults occupy an increasingly greater percentage of the population,[150] these chronic conditions will continue to become more important to understand and prevent. Making dietary changes may be one way to prevent the onset of these conditions. Dairy foods have long been considered nutrient-dense and health-promoting foods that offer many health benefits.[151,152] Whole milk is naturally rich in minerals (calcium, potassium, magnesium, phosphorus, selenium, zinc), vitamins (riboflavin, thiamin, A, B12), high-quality protein, carbohydrate, and fat.[6,153] In addition to these well-known macro- and micronutrients, recent evidence suggests that dairy foods contain other components that may benefit health outcomes as well, such as bioactive peptides,[154] the milk fat globule membrane (MFGM),[155] prebiotics,[156] and probiotics.[157]

Because the effects of dairy food consumption on chronic health conditions have been studied extensively, the literature in this review is limited to human studies conducted with free-living adult populations since 2009. The studies are written in English and primarily use whole dairy products instead of isolated nutrients or dairy product constituents. This review focuses on the dairy foods recommended for consumption 3 times a day by the U.S. Dietary Guidelines (yogurt, milk, and cheese).[44] In addition, all articles had to reference milk intake or dairy food consumption to be considered for this review. Data used in each category of health discussed (bone, cardiometabolic, cognitive, and digestive) originated from observational and intervention studies.

Bone Health

The treatment of osteoporotic fractures incurs billions of dollars in healthcare costs in the United States alone.[158] Osteoporosis, a multifactorial disease common in postmenopausal women, is characterized by decreased bone mass density (BMD) and increased bone fragility, bone porosity, and risk of fractures.[158] Osteoporosis is diagnosed when BMD measurements are equal to or less than 2.5 standard deviations below a "reference range" of healthy adult BMDs.[159] Osteopenia, or low bone mass, describes BMD measurements between 1 and 2.5 standard deviations below the healthy reference range.[159]

Adequate calcium intake is often recommended to prevent osteoporosis, because most of the body's calcium is stored in bones.[160] Sufficient calcium consumption during

adolescence, an important period of bone growth, may protect against bone mass loss in adulthood.[160] Because bone mass tends to decrease with age,[161] adequate calcium intake is highly recommended for postmenopausal women as well. The Inst. of Medicine (IOM) recommends 1300 mg of calcium daily for girls ages 9 to 18 and 1200 mg of calcium daily for women over the age of 51.[162] Despite these recommendations, the 2010 Dietary Guidelines (2010 DGA) and the 2015 Scientific Report of the U.S. Dietary Guidelines Committee (2015 DGAC) list calcium as a "nutrient of concern" for public health in the United States because of insufficient consumption rates.[44,93] Because dairy is naturally high in calcium, regular consumption of milk and other dairy foods is recommended, especially for adolescent and adult females.[44]

In addition to being high in calcium, dairy products also contain more utilizable calcium than most other foods. Calcium may be more bioavailable from dairy than from foods such as grains and leafy green vegetables, for instance, because dairy does not contain phytic acid or oxalates, which can bind calcium and prevent its absorption.[163] To be absorbed in the small intestine, calcium must be in either its "soluble ionized form (Ca^{2+}) or bound to a soluble organic molecule to cross the intestinal wall."[164] If low stomach acid prevents the complete dissolution of calcium before it enters the intestinal milieu, or if other molecules are not available to bind calcium, then calcium can precipitate and resist absorption in the alkaline environment of the small intestine.[164] In general, precipitation of calcium salts at this stage decreases calcium's solubility and absorbability.

The location of calcium in milk's casein micelles keeps it soluble and protected from precipitation. In these micellar structures, calcium is bound to phosphorus and forms a colloid. Forty-five percent of the calcium in milk is found in colloidal calcium phosphate.[163] Furthermore, as dairy calcium is digested, it can bind to casein phosphopeptides (CPPs), bioactive peptides[165] formed during casein proteolysis, that allow for greater passive absorption of calcium.[166] Like the absence of phytates and oxalates, the location of colloidal calcium phosphate in casein micelles and the binding of calcium to CPPs during digestion may improve the solubility and bioavailability of dairy calcium.[166] Therefore, phosphorus in dairy enhances the calcium absorption possible from dairy foods,[163] and calcium and phosphorus together account for 80% to 90% of hydroxyapatite, the mineral component of bone.[167]

In addition to calcium and phosphorus, the nutrients vitamin D, magnesium, zinc, and potassium are also found in milk and are vital to bone health. These nutrients enhance the bioavailability of dairy calcium and contribute additional bone-building properties. Table 3-1 shows the contributions that a serving of milk can make towards the daily recommended intake for all of these bone health related nutrients.

Vitamin D, which is routinely added to milk in the U.S., Canada, and some countries in the European Union,[168] is integral to calcium uptake and homeostasis and is vital to bone formation and health. Vitamin D assists with the active transport of calcium in the intestine and helps maintain serum calcium levels.[163] As with underconsumption of calcium, too little vitamin D also exacerbates osteoporosis risk by reducing calcium absorption. The IOM recommends consumption of 600 IU of vitamin D daily by children

and adults under the age of 70.[169] Consumption of 800 IU of vitamin D is recommended for adults over the age of 70. Unlike calcium, vitamin D can also be produced endogenously with sunlight exposure. The IOM values are intended for individuals who receive little such exposure. Older adults have higher vitamin D recommendations, because endogenous production of vitamin D also declines with age.[169,170] In the United States, milk is fortified with 100 IU of vitamin D per cup of milk.[171]

Dairy foods naturally contain small amounts of magnesium and zinc, which are also vital for bone health. Most of the magnesium in the human body is found in bone, and magnesium serves an important role in calcium homeostasis through its regulation of serum calcium levels and secretion of parathyroid hormone.[167] Magnesium deficiency may contribute to osteoporosis, and, like calcium and vitamin D, magnesium is an underconsumed nutrient in the U.S.[93,167] Zinc, on the other hand, primarily functions as an enzyme cofactor, but like calcium and phosphate, also forms part of the apatite portion of bone.[167] Low levels of zinc may be related to osteoporosis, but, according to the DGA, the intake levels of both magnesium and zinc are not currently a cause for public health concern.[93]

Although commonly represented as a valuable nutrient for blood pressure maintenance, potassium is also important for bone health and is present in dairy products.[93,172] Potassium contributes to bone health by assisting with calcium retention and preventing bone resorption,[173] and adequate potassium intake has been associated with higher BMD in adults.[174,175] Like calcium, potassium is considered a “nutrient of concern” in the U.S. due to habitually low consumption.[93,172] Americans consume just

56% of the adequate intake of potassium (4.7 g daily for most adults).[93,176] Potassium is not found in significant quantities in all dairy products, but both milk and yogurt qualify as "good sources of potassium" according to FDA guidelines.[176,177]

In addition to these nutrients, the lactose in milk may also influence calcium absorption and, in that way, influence bone health;[166] however, the specific role of lactose in calcium absorption remains poorly understood.[163] Animal studies suggest that lactose extends calcium retention time in the intestine and may, therefore, give the body more time to absorb calcium.[178] According to a review of calcium bioavailability, lactose is generally considered to "increase the passive absorption of calcium."[163] Studies of calcium absorption in infants consuming soy formulas with either glucose polymers or lactose added as the primary carbohydrate show that the infants consuming lactose formulas absorbed more calcium.[179] Yet, despite this possible benefit of lactose to calcium absorption, lower-lactose dairy foods, including yogurt and cheese, do not seem to affect calcium absorption.[163] More research is needed to determine the mechanism of action for lactose's impact, if any, on calcium absorption.

Finally, dairy foods are also a source of bioavailable protein, another vital component for healthy bones. Adequate protein intake is necessary for strong bones, because protein forms the matrix for bone upon which mineralization occurs.[167] In the U.S. the Recommended Dietary Allowance (RDA) for protein for adults is 0.8 g/kg body weight.[180] However, the effect of high amounts of dietary protein (2 g/kg or more) on bone remains largely unknown.[181] While greater protein intake increases urinary calcium excretion, it also increases intestinal calcium absorption.[181] Although some publications

have suggested that dairy protein intake disturbs bodily acid-base balance and results in bone resorption and calcium depletion,[182] several recent studies, including a meta-analysis, show that increased calcuria with intake of animal protein is not causal evidence of bone loss.[183–185]

Although the exact effects of high protein intake on bone have not been identified, prospective cohort studies suggest that an above-adequate protein intake may exert a slight positive effect on BMD in older adults.[186,187] A prospective cohort study tracked protein intake and BMD changes over 4 years in 615 older adults (mean age of 75). This group consumed an average of 68 g protein a day, but individuals in the lower 2 quartiles of protein intake had significantly more BMD loss at the bone and spine.[186] In another prospective cohort study of 572 women and 388 men ages 55 to 92, animal protein consumption was associated with increased BMD in women.[187] However, this association between protein intake and BMD was "negligible" in women consuming 1800 mg or more of calcium.[187] This study reported no association between animal protein and BMD in men, and no association between protein consumption and rate of bone loss. Results from the Framingham Offspring Cohort Study, which includes data from 3656 adults, suggest that combining animal protein and over 800 mg per day of calcium may protect against hip fracture.[188] However, this study also suggested that pairing a lower calcium intake with higher amounts of animal protein may increase the risk of hip fracture. Higher protein intake may contribute to BMD maintenance, but the results of these studies suggest that calcium and protein intake should both be considered in the diets of individuals at risk of osteoporosis.[188]

As an important source of both calcium and protein, dairy foods may be an especially important food group to consume for BMD maintenance in later adulthood.[188] To assess the connection between dietary calcium and protein on bone resorption, an intervention study assigned 50 overweight adults to consume either 1.2 g/kg dairy protein (or the equivalent from "mixed protein" sources) for 12 weeks as part of a hypoenergetic weight loss diet.[189] The dairy protein group had a significantly lower increase in the bone resorption marker deoxypyridinoline (P=0.008) in comparison to the "mixed protein" group.[189] The results of this study suggest that the nutrient profile of dairy products may act as protection from the weight-loss-induced bone resorption. However, these results come from a single intervention study. Much more research needs to be conducted on protein and bone health, and on dairy protein and bone health, to ascertain the specific effect of different dietary components on BMD.

Extensive research on the effects of dairy product consumption on bone health has been conducted, yet the mechanism or nutrient linking the two has not been definitively identified. Many studies attribute dairy foods' impact on bone health to its naturally high calcium content.[161] However, though the calcium in dairy items contributes to its positive association with bone health, recent publications assessing the effects of dairy products on bone health make inconsistent conclusions about the impact of dairy foods on BMD. The literature was identified through a PubMed search using the terms "bone health," "bone mass density," and "dairy," limiting results to articles with full texts available that were published within the last 5 years. Six studies published from 2013 to 2015 were reviewed. Because BMD and osteoporosis incidence involves dietary habits during

adolescence and adulthood, one study involving adolescent females has been included in this section of the review.

Recent cohort studies show positive benefits from dairy consumption on bone health. BMD and food frequency questionnaire (FFQ) data collected from over 2,500 participants in the Framingham Offspring Study show that intake of milk, yogurt, and other fluid dairy products, but not cheese and cream-based products, was associated with increased hip BMD.[188] The highest quartile of dairy consumers, who consumed an average of 1,110 ± 492 mg of calcium per day, also had the highest hip and spine BMD values (*P* trend: 0.001 and *P* trend: 0.02, respectively).[188] A cohort study of 625 middle-aged Polish women found that the women's femoral neck and hip BMD both positively correlated with total dairy calcium ($P<0.0048$, $P<0.0198$) as well as calcium from milk ($P<0.0039$, $P<0.0361$).[190] However, both BMD measures had a negative correlation with "dairy dishes," a term that was not defined in the study.[190] Women who had experienced a hip fracture tended to consume less calcium. In both studies, intake of fluid dairy (milk, yogurt) correlated with higher hip BMD, suggesting that the calcium levels of dairy, which tend to be higher in and more easily absorbed from fluid dairy products, may be responsible for this effect on BMD.

Similarly, cross-sectional studies of Asian adults, who typically consume little dietary calcium, found significant associations between dairy intake and improved bone health, suggesting that even small amounts of dairy could be beneficial. Elderly Japanese men (n=1479) who drank a single daily glass of milk had higher areal bone mineral density and lower levels of bone turnover than men who drank less milk.[191] The men

consuming one glass of milk each day consumed a mean of 577 ± 159 mg calcium and 9.8 ± 6.2 mg vitamin D.[191] The Korean National Health and Nutrition Examination Survey (KNHANES) results from 9,444 adults found that both dietary calcium and vitamin D status impacted osteoporosis incidence in a population with typically low calcium and dairy food intake.[192] As calcium intake and serum vitamin D levels increased in this group, osteoporosis risk decreased significantly. The highest quartile of dairy consumers in KNHANES consumed 413.2 ± 6.8 mg calcium daily and had serum vitamin D levels of 20.1 ± 0.3 ng/mL. Dietary vitamin D intake was not evaluated in the KNHANES study. In the KNHANES study, the highest quartile of dairy consumers had one serving of dairy per day, and more than 90% of the elderly Japanese subjects in Sato's study consumed one or less than one cup of milk per day. A single daily serving of milk, however, impacted BMD and osteoporosis risk in both studies.

Two additional cohort studies showed no associations between dairy consumption and bone health or even calcium consumption and bone health. A prospective observational study of 1,898 Dutch fracture patients and healthy U.S. patients found that both groups consumed similar amounts of calcium and calcium-rich foods (primarily dairy), despite that 1 group consisted entirely of fracture patients with osteopenia.[193] A prospective cohort study of 1,007 Portuguese adolescents also found no associations between BMD and dietary patterns evaluated at age 13 with BMD at age 17.[194]

Most of the recent evidence suggests the existence of some link between dairy consumption and bone health, but the reasons for this link remains unclear. These studies were conducted in populations living in different geographical areas. While 1 serving of

dairy may increase BMD in South Asian populations, where dairy consumption is typically low, populations in Western countries may need to consume additional dairy to experience similar benefits. Furthermore, not all of the studies controlled for the calcium content in the dairy foods consumed. Sato and others[191] even found that adjusting for calcium intake made the association between milk consumption and femoral neck BMD insignificant. Finally, in the majority of these studies,[188,190–193] the study populations consumed significantly less dietary calcium than the recommended amount. In Western countries with dietary guidelines, calcium requirements tend to be similar to the levels recommended in the U.S. (1100-1200 mg per day).[169] In Japan, however, 600 mg is the daily recommendation.[195] Dairy may benefit BMD in populations with habitually low calcium intake, but the possible impact of additional dairy or calcium consumption on BMD in the U.S. and Europe remains unclear. It also remains difficult to separate the effect of an increase in dietary calcium from an increase in dairy consumption, because many of these studies assessing the impact of dairy on bone attribute its benefits to the calcium content.

Sarcopenia

Another factor that can increase the risk of osteoporotic fractures is sarcopenia, or decreased muscle mass, strength, and performance. Like osteoporosis, sarcopenia is fairly common among older adults and is estimated to affect more than 50 million people.[196] Unlike osteoporosis, however, no universal definition of sarcopenia has been

determined.[196,197] A 2010 report from the European Working Group on Sarcopenia in Older People, however, suggests that muscle mass, hand grip strength, and gait speed measures more than 2 standard deviations below a mean reference value for healthy adults may be an appropriate diagnostic criterion.[196] As with bone resorption, some loss of muscle mass and strength naturally occurs with age, and sarcopenia and osteoporosis tend to be closely linked. A recent review surveyed the literature linking sarcopenia with osteoporotic hip fracture risk, concluding that there is a significant relationship between the incidence of osteoporosis and sarcopenia in older adults.[198] In most of the reviewed studies, osteoporosis was accompanied by sarcopenia, and sarcopenic individuals were more likely to experience a fracture.[198] Another review article proposed that the term "sarco-osteopenia" be adopted to describe individuals at risk of fracture because of muscle and BMD loss.[199]

The dietary interventions recommended to prevent or slow the progression of these 2 conditions vary. Although the connection between protein intake and BMD remains unclear, nutrition interventions to prevent sarcopenia primarily center upon increased dietary protein. Protein consumption above the RDA, such as 1 to1.5 g/kg bodyweight, has been suggested for older adults to "reduce the progressive loss of muscle mass with aging."[200,201] Protein supplementation may be 1 way to easily and effectively increase the protein intake of older adults. However, encouraging older adults to consume the recommended amounts of dairy foods (3 cups daily)[3] may also help increase the protein intake of this population. While dairy foods contain fewer grams of protein per serving (3 g/100 g) than meat, poultry, or beans, which have 26 g, 31 g, and 7.8 g protein

per 100 g, respectively, dairy protein contains all 9 essential amino acids and is both bioavailable and digestible.[6,24,202] In addition, dairy is also an affordable source of protein, an important consideration for many older adults.[24,202,203]

Few studies, however, have been conducted on the association between dairy product intake and lean muscle mass maintenance. A single blind randomized control trial of 100 adults ages 60 and older assigned participants either to follow their regular diet or to add 70 g of ricotta cheese at breakfast, lunch, and dinner, a total of 210 g daily, to their regular diet. The researchers estimate that the addition of ricotta increased participants' protein intake from 0.9 g/kg to 1.2 g/kg.[204] After 12 weeks, the participants in the ricotta cheese group had significantly better appendicular skeletal muscle mass (P=0.009), suggesting that consuming more dairy protein may contribute to the preservation of lean muscle mass in older adults. However, this study's participants were healthy adults, and the results of this study can only be applied to individuals without sarcopenia. Consumption of more protein, especially from high-quality sources such as dairy, may help prevent sarcopenia but may not ameliorate it. Given the growing number of older adults, many of whom will likely suffer from osteoporosis, sarcopenia, or both, studies on dietary prevention strategies for both of these conditions could make a considerable impact on the lives of many older adults.

Cardiometabolic Health

The term "metabolic syndrome" describes a cluster of risk factors: central obesity, high triglyceride level, high blood pressure, insulin resistance, inflammation, and a

prothrombotic state. All increase the risk for other health problems, especially type 2 diabetes and cardiovascular disease. According to national survey data, 34% of American adults have metabolic syndrome,[205] and public health studies indicate that this number is likely to rise.

Because the National Health Statistics Report[205] listed central obesity, high blood pressure, and hyperglycemia as the most common risk factors for metabolic syndrome, this review will focus on the connection between dairy consumption and these 3 risk factors. The definition for clinically significant central obesity, blood pressure, and fasting glucose values are listed in Tables 3-2 and 3-3.

Central Obesity

In addition to being a component of metabolic syndrome, central obesity is associated with insulin resistance, higher mortality risk, diabetes, and adverse cardiovascular symptoms. Central obesity, or abdominal obesity, is defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) as waist circumference values greater than 88 cm for women and 102 cm for men.[205] Different organizations, however, including the International Diabetes Federation (IDF) and the World Health Organization (WHO) have proposed different clinical definitions of central obesity for populations of different geographical areas and ethnicities (Table 3-3).[206] To assess the impact that dairy has on central obesity, the search terms “central fat,” “abdominal obesity,” and “dairy” were used in PubMed to identify the recent literature

sources on this topic. Results from the 5 studies identified with these search terms are discussed below.

Two of the recent studies identified[207,208] found positive associations between the consumption of high-fat dairy foods, including whole fat milk, cheese, yogurt, cream, and butter, and a decreased risk of central adiposity. Both studies adjusted for potential confounders including physical activity, education, age, and alcohol intake prior to analysis. A prospective cohort study of 1,405 rural men ages 40 to 60 years found that men with a high dairy fat intake from whole milk, butter, and cream were less likely to develop central obesity at the 12-year follow-up time point than men with medium or low intakes of dairy fat.[207] Dairy fat intake was assessed by typical choice of sandwich spread (butter, low-fat margarine, fat-free margarine), milk (full-fat, 1.5% fat, skim milk), and cream consumption (daily, sometimes, seldom/never).[207] Men who chose butter and whole milk and consumed cream daily or sometimes were identified as "high dairy fat consumers," while the "low dairy fat consumers" avoided butter, selected low-fat or fat-free milk, and seldom or never ate cream.[207] Men who consumed any other combinations of these items were categorized as "medium dairy fat consumers."[207] This study defined abdominal obesity by a waist-to-hip ratio greater than or equal to 1. While the "high dairy fat consumers" were less likely to develop central obesity, the "low dairy fat consumers" were more likely to develop central obesity. Similarly, cross-sectional data from 1,352 adult participants of the Observation of Cardiovascular Risk Factors in Luxembourg showed that individuals who consumed high-fat milk, cheese, or yogurt had lower rates of global and abdominal obesity even after adjustments for potential confounding

variables.[208] Waist circumference was lowest in individuals with the highest whole fat dairy intake, and overall, "higher total dairy food intake was significantly associated with a lowered prevalence of global and abdominal obesity, by up to 50%."[208] In this study, abdominal obesity was defined using the NCEP ATP III definition.

Two additional studies, a crossover study and a controlled feeding study, found less promising results. Dugan's crossover study of 33 adults with metabolic syndrome found that the consumption of low-fat dairy as a control snack instead of a granola bar control led to lower waist circumference only in women.[209] However, in this study, the carbohydrate and dairy foods were isocaloric but not matched for nutrient content. The dairy snack had more protein and calcium and less carbohydrate than the carbohydrate control. Although Dugan's crossover study did show some, if inconsistent by gender, results, a controlled 15-week feeding study of hypocaloric diets in 71 overweight adults found that a high-dairy group provided with up to 4 servings a day of dairy foods experienced no changes in weight, fat, or intra-abdominal adipose tissue compared to the control low-dairy group.[210] Like Dugan's study, this controlled trial from Van Loan and others[210] used primarily low-fat dairy (with the exception of full-fat cheese). The evidence from Holmberg and others[207] and Crichton and Alkerwi[208] suggests that the fat content of dairy may be associated with its effect on central adiposity. Therefore, the results of Dugan and others and Van Loan and others may be attributable to the low-fat dairy foods used in the studies.

Finally, in a 9-year prospective study of 3,417 adults that did not distinguish between low-fat and high-fat dairy consumption, men and women with a higher dietary

calcium intake had a "lower increase in waist circumference."[211] The calcium levels of different dairy foods, in addition to their fat content, may have an impact on central adiposity. Some high-fat dairy products, especially hard cheeses, actually have an even higher calcium content than fluid milk,[153], which may contribute to the discrepancies in the results of these studies. Dietary calcium may contribute to the precipitation of long-chain fatty acids, prevent their absorption in the intestine, and increase their excretion.[164,212] Less fat absorption could contribute to weight control.[164,212] The precipitation of long-chain fatty acids by calcium suggests a possible mechanism for dairy's impact on central adiposity. However, more research is needed on this mechanism. The observed inverse association between dietary calcium and central obesity could also be a result of other components present in dairy products.

Another component in dairy foods that may contribute to this association, especially in studies showing a connection between dairy fat consumption and obesity, is conjugated linoleic acid (CLA). CLA is a trans fatty acid naturally present in dairy and beef. When provided as a supplement in animal studies, CLA contributed to reduced fat mass.[213] A meta-analysis of human supplementation trials using CLA showed that 3.2 g daily of either mixed CLA isomers or purified *trans*-10,*cis*-12 CLA isomers accelerated fat loss compared to placebo treatments ($P<0.001$).[213] However, this meta-analysis found no dose-response effect in the studies reviewed. Subjects receiving 6.8 g/d of CLA in supplements, instead of a dose of 3.4 g/d, did not lose more weight with the higher dose. In addition, most of the studies included in the meta-analysis had fairly short durations of no more than 12 weeks.[213] Finally, though the fat loss in these trials was attributed to

CLA, the CLA was provided as a concentrated supplement rather than in dietary forms. The supplement form of CLA is typically a mixture of 2 common isomers: *cis*-9,*trans*-11 and *trans*-10,*cis*-12.[213] However, 75 to 90% of the CLA in dietary sources is the *cis*-9,*trans*-11 form.[214]

The CLA content of dairy and beef products can vary significantly based on production method, season, and geographical location. CLA is typically only present in small amounts in dairy products (from 0.0007 g to 0.0227 g per 100 g of whole milk), but is present in much higher concentrations in "organic" than in conventional dairy items.[214] Organic milk has about 18% more CLA than conventional milk, according to an 18-month study of the composition of organic versus conventional whole milk.[214] These differences in CLA content are believed to be due to different feeding practices between "organic" and "conventional" cows. In the United States, organic dairy cattle feed must contain 30% pasture grasses and legumes for at least 120 days a year. Pasture-feeding influences the microbial population in the rumen of dairy cattle, which hydrogenates polyunsaturated fatty acids and stimulates CLA production.[214] CLA content of milk also depends on the season and the milk's region of origin. While organic milk overall has more CLA than conventional milk ($P<0.001$), organic milk from the northeastern region of the U.S. has even more CLA than organic milk from other U.S. regions ($P<0.001$). CLA levels in organic milk are also about 55% higher in the summer than in the winter ($P=0.000000$).[214]

One human feeding study assessed the health benefits of organic versus conventional dairy food in terms of CLA content in 18 healthy 20- to 39-year old-

women.[215] These women were randomly divided into 2 groups, one of which consumed pasture-fed dairy and beef products in their meals and the other consumed grain-fed dairy and beef. All other aspects of their diets remained the same. The only discernable difference farming technique had on the dairy and beef items in terms of nutrition was CLA content. Women consuming the pasture-fed products consumed 1.17 g per day of CLA, while the grain-fed dairy and beef group consumed only 0.35 g of CLA daily. After 8 weeks on these diets, however, the CLA did not affect the body composition, blood lipids, or insulin sensitivity of the subjects. Although this study had a relatively small sample size and short duration, its results suggest that CLA from dietary sources may not affect common risk factors of the metabolic syndrome. Given the results of the meta-analysis on CLA supplementation, however, further study on both CLA supplementation and CLA from natural sources is warranted to assess its potential to impact body composition and metabolic syndrome prevention.

Elevated Blood Pressure

High blood pressure is a prevalent component of the metabolic syndrome among the U.S. population and causes more deaths than other cardiovascular risk factors.[216] According to the Eighth Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, high blood pressure is defined as 140/90 mmHg for the general population younger than 60 years old and 150/90 mmHg for the general population over the age of 60.[217] Among the associations of metabolic syndrome parameters to dairy consumption, the connection between high dairy intake and

normal blood pressure seems to be the strongest. The potential mechanisms behind this association are supported by results of recent observational and intervention studies.

Part of the proposed explanation for dairy's effect on lowering blood pressure is its micronutrient content. Dairy contains calcium, vitamin D, potassium, and magnesium, all of which have been associated with blood pressure regulation.[218] When calcium levels are low, levels of 1,25-dihydroxyvitamin D (the biologically active form of vitamin D) increase and upregulate calcium flux into cells.[218,219] When calcium uptake occurs in smooth muscle cells, it exerts a vasoconstriction effect that increases blood pressure.[216,218] However, dietary calcium may mitigate this vasoconstriction effect of 1,25-dihydroxyvitamin D by enabling the body to maintain serum calcium levels more easily.[216,218] Serum calcium is typically carefully regulated, but with chronically low dietary intake or poor intestinal absorption, serum levels will fall and then calcium will be taken from bones to maintain regular serum levels.[161] Therefore, dietary calcium maintains bone health by preventing the uptake of calcium from bones in addition to regulating blood pressure. As one of the most important sources of calcium in the diet,[220] dairy products should act to lower blood pressure.

In addition, dairy proteins digested via enzymatic proteolysis or fermentation release bioactive peptides that act as vasodilators or angiotensin-converting enzyme (ACE) inhibitors. Casein and whey proteins contain casokinins and lactokinins, respectively, which function as vasodilators. Casokinins include the lactotripeptides Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP) and significantly lowered participants' blood pressure in intervention trials.[218,221] Similarly, the lactokinins α-lactalbumin and β-

lactoglobulin release bioactive peptides that function as ACE inhibitors. ACE inhibitors and ACE inhibitor potency is measured by the concentration needed to lower 50% of total ACE activity (IC_{50}),[154] therefore, lower IC_{50} values indicate greater potency. Generally, bioactive peptides in dairy have a much lower antihypertensive potency than synthetic ACE inhibitors. Synthetic ACE inhibitors, often prescribed to treat hypertension, typically have an IC_{50} of 0.02 μM.[154] The strongest ACE inhibitor in dairy, ALPMHIR, has an IC_{50} of 43 μM.[154]

However, the enzyme used to hydrolyze dairy proteins affects the potency of dairy's ACE inhibitory functions.[154] Enzymatic hydrolysis with trypsin seems to generate the lowest IC_{50} values with dairy peptides,[154] and further research in this area may generate additional processes to increase the potency of ACE inhibitors in dairy. Despite the promise of these bioactive dairy peptides as antihypertensive agents, they seem to be less effective *in vivo* than *in vitro*.[154] Further research in human populations is needed to explore the possibility of dairy peptides as vasodilators. However, results from 2 recent reviews[216,218] and 5 primary research articles[211,222–225] found via PubMed searches for "blood pressure," "hypertension," and "dairy" suggest that, regardless of the mechanism, consumption of dairy foods may lower blood pressure.

Although in these trials dairy consumption improved only systolic or diastolic blood pressure, most of these studies did show a positive impact of dairy intake on blood pressure. Fumeron's prospective study on metabolic syndrome markers in 3,417 French adults found that dairy and dietary calcium intake were associated with lower diastolic blood pressure in all participants, but were only associated "with a lower increase in

systolic blood pressure" in men.[211] Another publication on the same prospective study found that "calcium density" was also "associated with a lower systolic blood pressure" in all participants.[222] Similarly, an observational study of healthy French-Canadian adults (n=233) did not find associations between total dairy food intake and blood pressure, but did find that high-fat dairy food intake was related to lower diastolic blood pressure in men, while low-fat dairy food intake was related to lower systolic blood pressure in women.[225]

However, 2 crossover studies with subjects who consumed either low-fat dairy items or a nondairy snack for several weeks had contrasting results. Van Meijl and others[223] found that the 35 overweight or obese adult subjects in their study had lower systolic blood pressure after 8 weeks of dairy food consumption, while Maki and others[224] found that the 62 adults with pre- or stage 1 hypertension had no significant change in blood pressure after 5 weeks of dairy food consumption. Potential reasons for the differences in these studies could be a result of the differing intervention times or the geographic location of these studies. Van Meijl's cohort was comprised of healthy Dutch adults while Maki's subjects were from the midwestern U.S. Furthermore, Maki's subjects had high blood pressure at the beginning of the study unlike the other studies that assessed for changes in blood pressure changes in healthy adults over time. While dairy seems to exert a protective effect on blood pressure regulation, it may not ameliorate pre-existing hypertension.

Dietary Approaches to Stop Hypertension

Yet, though these recent studies fail to show a decrease in blood pressure with dairy food consumption, low-fat and fat-free dairy foods are integral components of the Dietary Approaches to Stop Hypertension (DASH) diet.[226] Developed in the mid-1990s, the DASH diet is recommended by the National Heart, Lung, and Blood Institute (NHLBI) as an eating plan for the prevention or treatment of hypertension.[227–230] The DASH diet promotes consumption of nutrients and foods beneficial to heart health and blood pressure, especially potassium, calcium, fruits, vegetables, and dairy products, and avoidance of sodium and saturated fat.[226] The DASH diet has been shown to lower systolic blood pressure by 8 to 14 mmHg.[231]

The DASH diet was developed with input from two major clinical studies, the DASH study[227] and the DASH-sodium trial.[228,230] The initial DASH study focused on the effects of 3 different eating plans- a typical American diet with low fruit, vegetable, and dairy product intake, a diet with an average of 9 total vegetable and fruit servings, and a diet with 9 fruit and vegetable servings as well as 2 servings of low-fat dairy products on blood pressure.[227] Sodium intake was held constant for all of the diets, and participants (n=459) were regularly weighed to ensure no weight loss during the trial. The diet that included low-fat dairy as well as fruits and vegetables resulted in significantly lower blood pressure ($P<0.001$) compared to both the fruit and vegetable-rich and typical American diet plans. In a follow-up to this DASH trial, the DASH-sodium trial assessed the effect of modulating sodium intake on blood pressure in both the DASH diet and a typical American diet. This trial found that the DASH diet lowered blood pressure at all

sodium intakes (3.5 g, 2.3 g, 1.2 g), but a combination of the DASH diet and reduced sodium intake (1.5 to 2.3 g sodium) was most effective for hypertension control.[228,229]

Although the original DASH diet did not aim to specify the components in the diet tied to its effectiveness nor did it assess the impact of long-term consumption of such a diet, its results suggest that low-fat dairy may be an integral component to nutrition interventions to prevent and treat hypertension. As a result of these trials, a DASH diet paired with low sodium intake is the current recommendation for blood pressure regulation through diet.[226] Yet, results of a recent study suggest that, based on food frequency questionnaires and blood pressure ratios of 2,187 adults from 1991 to 2008, a DASH-type diet may not be effective for long-term reduction in blood pressure.[232] Therefore, while the DASH diet can be recommended as a health-promoting diet for short-term blood pressure regulation, its long-term effectiveness has not been evaluated extensively. More large studies are needed to assess the long-term efficacy of the DASH eating plan.

Hyperglycemia

Hyperglycemia, a third major risk factor for metabolic syndrome, is also an important risk factor for type 2 diabetes and coronary heart disease. Clinically significant hyperglycemia is defined by fasting glucose levels greater than or equal to 110 mg/dL, where normal ranges for fasting glucose are 70 to 100 mg/dL.[205] According to the American Diabetes Association, “prediabetic” describes fasting glucose levels between 100 and 125 mg/dL, and fasting blood glucose levels of 126 mg/dL or higher indicates

diabetes.[233] In comparison to the other two parameters of the metabolic syndrome discussed above, less research has been conducted on the effects of dairy products on fasting blood glucose levels.

A recent review [218] proposes that the effects of dairy foods on blood glucose levels are a result of the protein content of milk and its insulinotropic properties, which may decrease serum glucose levels. Replacing carbohydrate with protein, for instance, may shift the body to gluconeogenesis and stabilize glucose concentrations.[218,234] Additionally, and importantly for type 2 diabetics, consuming protein with carbohydrate may help the body to secrete more insulin and, therefore, exert more control over blood glucose.[218] The amino acid profile of dairy peptides may be especially helpful for milk's ability to mitigate an *in vivo* glucose response.[225]

The fatty acid content of dairy foods may also influence insulin response. Dairy fat is one of the only sources of the unsaturated fatty acid *trans*-palmitoleic acid, which has been associated with lower insulin resistance ($P<0.001$) and a lower incidence of diabetes in a cross-sectional study of 3,736 adults.[235] A study with 17 adults reported similar findings.[236] However, a third cross-sectional study of 85 adults did not find a connection between *trans*-palmitoleic acid and insulin sensitivity.[237] As with CLA, milk production methods lead to significant variations in the *trans*-palmitoleic concentrations of milk,[214] which may account for the different results of these studies if study subjects consumed different types of milk. Besides *trans*-palmitoleic acid, other fatty acids in ruminant and dairy fat, such as heptadecanoic acid and pentadecanoic acid, may also be associated with insulin sensitivity.[236,237] All of these fatty acids, however, are present in

very small amounts in dairy foods and have typically been used in research as biomarkers of dairy fat presence and consumption. Although cross-sectional research indicates a possible association, given the small amounts of these fatty acids that people typically receive from dairy foods, it is difficult to determine their specific impact, if any, on health without conducting intervention studies.

Five of the studies used for this review that discuss associations between dairy food consumption and central obesity or hypertension also address the association between insulin resistance and dairy food consumption. The results of these studies suggest that inadequate evidence exists to conclude that dairy food has a significant effect on hyperglycemia. While some studies, including a controlled feeding study[210] and a randomized control trial,[223] found no effect of dairy food intake on fasting blood glucose levels, a prospective cohort study of 3,435 French adults found that dairy product consumption (other than cheese) and dietary calcium were inversely associated with "lower incidence of metabolic syndrome and impaired fasting glycemia and type 2 diabetes" after 9 years.[211] Furthermore, in women, dietary calcium was negatively associated with insulin levels, which contrasts the insulinotropic theory of milk's effect on fasting glucose levels.[211] The authors hypothesized that this difference is due to the chronic versus acute effect of milk on insulin levels. A crossover study of 33 adults found that men, but not women, who had been diagnosed with metabolic syndrome had lower glucose levels after 6 weeks of low-fat dairy product consumption (P=0.048).[209] Yet, in an observational study of 233 Canadian adults, total and low-fat dairy product intakes were "inversely correlated with fasting plasma glucose level," but this relationship only

remained significant in women (P=0.007 and P=0.03) when the results were stratified by gender.[225] One additional study on 17 adults with nonalcoholic fatty liver disease did find an association between biomarkers of dairy fat consumption and glucose tolerance,[236] but the small size of this study as well as its very specific inclusion criteria limit its generalizability. Overall, these studies present little evidence regarding the impact of dairy on blood glucose and while dairy, especially dairy fat, may have an impact on fasting glucose levels, too little information can been compiled to support a definitive conclusion.

Cardiovascular Health Markers

Dairy foods may also exert a positive influence on cardiovascular risk factor markers. Specifically, certain fatty acids in dairy foods, including *trans*-palmitoleic acid, stearic acid, lauric acid, myristic acid, and oleic acid, have been associated with some beneficial effects on blood lipids and serum lipoprotein levels. Phospholipid *trans*-palmitoleic acid levels, also used as a biomarker for dairy fat presence or consumption, were associated with higher high-density lipoprotein cholesterol (HDL-C) (P=0.04), lower triglycerides (P<0.001), and a lower ratio of total-to-HDL-C (P<0.001) in a prospective cohort study of 3,736 older adults.[235] When compared to a carbohydrate replacement, consumption of dietary stearic acid, a saturated fatty acid amounting to about 12% by weight of milk fat,[238] reduced triglyceride levels (P<0.001) and total cholesterol levels.[239] A recent review, however, counters that this fatty acid has an overall neutral, but not detrimental, effect on serum lipids and lipoproteins.[240]

A 2003 meta-analysis used the results of 35 feeding trials to describe the effects of specific fatty acids, including lauric acid and myristic acid, on serum lipoprotein levels. The results suggest that lauric acid, a saturated fatty acid that amounts to 3.3% of the fatty acid content in milk,[238] may increase low-density lipoprotein cholesterol (LDL-C) levels but raise HDL-C levels even more.[241] Myristic acid, also a saturated fatty acid, increased total, LDL-C, and HDL-C levels, but according to another review, it "does not affect total cholesterol:HDL ratio."[241]

Oleic acid, another potentially beneficial fatty acid in dairy, accounts for 23.8% of the total fatty acid content in milk.[238] Oleic acid, which makes up most of the fatty acid content in olive oil, has been associated with improved HDL-C and LDL-C levels in patients with hypercholesterolemia.[242] Although much more research is warranted on the effects of these different fatty acids on human health, all current data suggest that some fatty acids in dairy fat may offer health-promoting benefits to cardiovascular disease risk factors. More information is needed on these components and on their synergistic effects in dairy foods.

Cognitive Health

With the increase in life expectancy and an aging population, dementia rates are expected to rise.[243] Dietary factors may contribute to cognitive decline. A recent literature review states that cognitive health is impaired by metabolic syndrome parameters, especially hypertension and hypertriglyceridemia.[244] Although the reason for dairy foods' associations with improved cognitive health has not been definitively determined, it has

been attributed to the ACE inhibitors in their bioactive peptides[154,165,216,218,245–247] and the phospholipid content of the MFGM.[248] The antihypertensive effects of these bioactive peptides are described under the "Blood Pressure" heading of this review. Another review article suggests that the phospholipid content of the MFGM may benefit cognitive health and delay the onset of Alzheimer's disease.[248] Each milk fat globule is comprised of a triglyceride core coated with phospholipids and proteins and surrounded by a membrane bilayer "derived from the apical surface of the mammary epithelial cell."[249] This bilayer is the milk fat globule membrane. However, few studies have been performed to assess the cognitive health effects of the MFGM, and a PubMed search for intervention trials involving the effect of MFGM on cognitive health in humans generated only a single study. In this study, infants who consumed MFGM-supplemented formula until 6 months of age had significantly higher cognitive scores (P=0.008) at 1 year of age than the infants fed a standard formula diet.[155] There was no statistical difference between MFGM formula-fed infants and the control breastfed infants.[155] Overall, the mechanistic link between cognitive health and dairy consumption lacks a strong evidence base.

However, dairy foods do have an epidemiological association with improved cognition. Three recent observational studies show associations between improved cognitive health and dairy product consumption. Cross-sectional data from 1,183 middle-aged South Australian adults showed some significant associations between low-fat dairy intake and improved social and cognitive health.[250] Improved social functioning was associated with low-fat yogurt consumption in men (P=0.045) and with low-fat cheese consumption in women (P=0.021).[250] Low-fat yogurt consumption in men was also

associated with better memory recall (P=0.029). Whole fat dairy consumption was associated with poor cognitive and psychological health. Cross-sectional data from 972 adults in the Maine-Syracuse Longitudinal Study (MSLS) also showed that adults who consumed 2 to 4 servings of dairy foods a week performed better on cognitive tests than adults consuming just 1 weekly serving of dairy.[247] Data from the National Health and Nutrition Examination Survey also show positive correlations between short-term memory assessment scores and total dairy consumption (P<0.0001) as well as between higher story recall scores and cheese consumption among adults 60 years old and older (P<0.0001).[251] Additionally, these authors hypothesized that dairy food consumption may have a "threshold effect" on cognition among older adults, because the difference between dairy consumers and non-consumers was greater than the differences between groups of dairy consumers. This information suggests that the effects of dairy products on cognition may be affected by long-term consumption habits.

While cross-sectional studies show correlations between dairy consumption patterns and improved cognitive health, the single intervention study assessing the effects of dairy on cognition showed few changes in cognitive performance with dairy consumption. Based on a literature search conducted in PubMed, only 1 intervention study assessing the effects of dairy consumption on cognitive health has been published. Crichton and others[252] assessed the cognitive effects of 6 months on a high-dairy diet (4 daily servings of reduced-fat dairy products, including milk, yogurt, and custard) compared to a low-dairy diet (1 or fewer daily servings) in 38 overweight adults who typically consumed little dairy. This study found that the diet high in dairy foods had a

minimal impact on the cognitive measures used, which included "10 neuropsychological tests and 1 questionnaire assessing psychological well-being." After 6 months of high levels of dairy food consumption, participants had slightly higher working memory scores (P=0.046), but no other measures showed significant changes following the high-dairy diet versus the low-dairy diet.

Because most studies linking cognitive health and dairy products rely on cross-sectional data, the reasons for improved cognition with high-dairy consumption may be due to confounding factors. Individuals with other healthy habits that lower chronic disease risk factors may be the same people who consume greater amounts of low-fat dairy products and experience less cognitive decline. In the MSLS study, for instance, Crichton and others[247] describe how the high-dairy consumers were also less likely to smoke and consume alcohol and were more likely to eat vegetables than the low-dairy consumers. These "healthy" habits have also been independently correlated with lower incidence of detrimental health problems, including type 2 diabetes, cardiovascular disease, and obesity, all of which have also been linked to impaired cognitive health.[246]

Digestive Health

The term "digestive health" covers a very broad range of conditions and symptoms, and it does not share the same quantifiable biomarkers and testable measures as cardiometabolic health, bone health, or even cognitive health. Digestive health is the only 1 of these 4 categories not addressed in the 2015 DGAC.[97] The American Gastroenterology Association describes good digestive health as "a digestive system that

has appropriate nutrient absorption, intestinal motility, immune function, and a balanced microbiota (the community of microorganisms that live in the gut)."[253] A literature search in PubMed with the key words "digestive health" and "dairy" yielded no results for studies relating overall digestive health to dairy products in lactase-persistent human populations. However, dairy foods, especially fermented products, contain prebiotics and probiotics that contribute to improved gut health through promotion of diversity and modulation of intestinal bacteria. Prebiotics are food components that resist digestion but are primarily fermented in the intestine and promote the growth of beneficial microorganisms including bifidobacteria and lactobacilli.[156,254] Probiotics have been defined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[255] Most of the probiotic- and prebiotic-containing foods on the market are dairy-based foods.[256]

Although most prebiotics are plant-derived, milk also contains prebiotic compounds. Galactooligosaccharides (GOS) are naturally present in small amounts in all mammalian milks and are also produced commercially for use as functional ingredients.[156,257] In 2001, the European Commission Scientific Committee on Food approved a mixture of GOS and fructooligosaccharides for use in infant formula[258] to mimic the beneficial prebiotic effects of the GOS present in breast milk. In infants, as well as adults, GOS have an established effect on the proliferation of beneficial gut microorganisms, especially in the colon.[156] Some of the gastrointestinal benefits of increasing bifidobacteria, lactobacilli, and other microbial populations in the colon include

protection from diarrheal diseases, inhibition of pathogenic infections, and amelioration of constipation.[156,157,256] GOS remain undigested in the stomach and small intestine but are fermented in the large intestine. This fermentation process may improve calcium absorption by decreasing the pH of the intestinal milieu, allowing for greater calcium solubility.[254] In a 3- week crossover study of 31 adolescent females given varying levels of GOS daily in supplemented smoothies, subjects had increased fecal bifidobacteria levels ($P<0.03$) and significantly improved calcium absorption.[259] However, though emerging research in this area seems promising, not enough data, specifically related to the GOS content of dairy foods and digestive health, exist to assess their effects at this time.

Dairy foods are the primary probiotic-containing food products in the marketplace.[157] The most frequently consumed probiotic dairy foods are yogurt, kefir, buttermilk, and other fermented milks, all of which contain benign or beneficial bacteria like lactobacilli and bifidobacteria.[157] The bacterial content of these fermented milk products can ameliorate symptoms of diarrheal disease and decrease symptoms of lactose intolerance, but definite evidence of other health benefits associated with probiotics has not yet been established adequately at this time;[157] however, research in this field is currently very active and promising (Professor M. Kroger, professional communication).

Lactose Intolerance

Although there were no studies on overall digestive health and dairy consumption in lactase persistent populations, some research has been dedicated to decreasing the

gastrointestinal (GI) symptoms induced by dairy food consumption in lactose intolerant individuals. Some strategies to treat lactose intolerance suggested in the literature include restriction of lactose-containing foods, use of lactose-reduced products, gradual increase of lactose-containing foods, frequent consumption of lactose throughout the day, consumption of lactose-containing foods with other nutrients, and use of probiotics.[260]

Lactose intolerant individuals produce insufficient beta-galatosidase (lactase), the enzyme needed to hydrolyze lactose into glucose and galactose so those individual monosaccharides can be absorbed in the small intestine.[157,260] Lactose intolerance can lead to several GI symptoms, including borborygmi, diarrhea, flatulence, and general discomfort when lactose-containing foods, including dairy products, are consumed.[157,260] Symptom severity depends on lactose intake, and one systematic review found that doses up to 12 g (the amount of lactose in 1 cup of milk) were fairly well-tolerated with minor or no adverse GI symptoms.[261] Doses between 15 g and 18 g caused more symptoms but were generally well-tolerated when consumed with other nutrients.[261] Doses greater than 18 g caused significant GI symptoms.[261]

Dairy products that contain less lactose due to lactic acid fermentation, such as yogurt (8.28 g lactose/cup) and cheddar cheese (0.30 g lactose/cup),[§§] are the source of disagreement in the literature regarding their digestibility by lactose intolerant individuals. One review suggests that lactose digestion can actually be improved with the

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[§§] Lactose amounts were calculated using Nutrition Data System for Research software version 2014 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

intake of fermented dairy products.[262] Additionally, another review mentions a study that found some symptom improvement in lactose intolerant individuals with supplementation of specific probiotic species (*Lactobacillus casei Shirota* and *Bifidobacterium breve Yakult*).[260] However, too little evidence currently exists to support a recommendation for lactose intolerant individuals to consume fermented and cultured milk products to improve lactose digestion and reduce GI symptoms.[260,261]

Lactose intolerance is relatively common in certain geographical areas, especially Italy (70% of the population), Central Asia (80%), and certain regions of Africa (70-90%).[260,262] Yet, considering its prevalence, there is relatively little data on treatment for lactose intolerance, and a 2010 systematic review found no studies on the long-term effects of lactose-exclusion diets on GI symptoms.[261] However, lactose intolerance is also difficult to study, because diagnosis and report of GI symptoms are largely based on self-report. Although lactose maldigestion, or the presence of unabsorbed lactose in the intestine, can be assessed with breath hydrogen testing, "demonstration of lactose intolerance relies on self-reported symptoms after lactose ingestion."[261] Considering that "inadequate dairy food intake may have adverse effects on health,"[151] lactose intolerance as a reason for avoidance of dairy products merits further research to ensure that lactose intolerant individuals receive adequate nutrients without excessive GI distress.

Limitations

Dairy consumption has evidence-based links to bone health and hypertension, and current epidemiological data linking cognitive health to dairy consumption seem

promising. Dairy foods may also exert protective effects against hyperglycemia and central adiposity and support digestive health. However, even the strongest associations between dairy and health had at least 1 study with contrasting results. These variations in data could be a result of inter-individual differences among study subjects, genetic factors, geographic location, or even gender. In addition, these studies all have limitations to their methods or analysis procedure that prevent the generalization of their results. Some study limitations include confounding variables, inaccurate subject reporting, and use of the umbrella term "dairy" to encompass a range of foods with different nutritional profiles, which could significantly impact their health effects.

Consumer Public Health Impact of Dairy Food Research

Despite the vast extent of research that has been conducted on dairy products and their connections to human health, including their associations with chronic disease risk and prevention, the U.S. Food and Drug Administration (FDA) has approved few health claims applicable to dairy products (Table 3-4). The contribution of calcium and vitamin D to osteoporosis prevention has been determined by the FDA as supported by enough evidence so that products high in these 2 nutrients can claim to support bone health.[263] Products qualifying for this claim must meet several criteria, including containing a minimum of 20% of the daily value of both calcium and vitamin D. Reduced-fat milk (2% and 1%) and skim milk meet the standards for this health claim.[264] Some dairy products also meet the required criteria to make FDA-approved claims based on sodium and hypertension, saturated fat and coronary heart disease, and potassium and blood

pressure. The European Food Safety Authority (EFSA) allows many more health claims for dairy foods due to their nutrient content, including claims related to blood clotting, metabolism, muscle function, and neurotransmission (Table 3-4). However, these health claims do not encompass the potential health benefits attributed to dairy food consumption (Table 3-5).

Although there is evidence of benefit from dairy food consumption, as suggested by FDA-permitted health claims, as well as current USDA recommendations to consume 3 servings daily,[93] more research is warranted to determine in more detail the contributions of dairy foods to health. Some naturally present components of dairy products discussed in this review, including GOS and probiotic cultures, are being produced and added to foods by the industry for nutritive or functional purposes. A review on applications for GOS in the industry, for instance, suggests that they can function as both a sugar or fat replacement in foods as well as prebiotics.[156] However, although these dairy-derived components are "generally recognized as safe" by the FDA, they are not supported by a strong enough evidence base to be health claims. The FDA requires human studies and proven prevention or risk reduction for certain diseases as a basis for health claims. To enable the emerging evidence of dairy foods' nutritive properties beyond macro- and micronutrient contents to be employed in industry and clinical practice, more human intervention studies with both dairy foods and individual dairy nutrients are warranted.

Dairy Nutrition and Consumer Trends

Dairy foods are already recommended for daily consumption by public health authorities in many countries (Table 3-6). Without consuming either dairy foods or calcium-fortified foods, most adults and children cannot consume enough calcium to meet recommended levels.[151,220] Given calcium's role in bone health and the normative functioning of other body systems (Table 3-4), consuming an adequate amount is important for health. However, at least in the U.S., individuals over the age of 5 do not meet the recommended intake for dairy foods.[73]

Pending additional research into the health-promoting properties of dairy foods' many components, current nutrition policies as well as consumer preferences can be used to formulate dairy products that are both health-promoting and palatable. Supplying foods that satisfy both consumer desires and dietary guidelines would be an invaluable way for the food industry to support public health. Some areas for industry to focus on in terms of dairy foods include added sugars, milk fat, protein, and cultured dairy products.

Added sugars, milk fat, and natural sweeteners

Policymakers around the world are encouraging people to decrease sugar consumption. In the U.S., the FDA proposed a "daily value" for added sugars in July 2015. This daily value would be listed on the Nutrition Facts label of all packaged foods and would recommend that added sugars be limited to 10% or less of total daily calories.[265] According to choosemyplate.gov, added sugars refers to "sugars and syrups

that are added to foods or beverages when they are processed and prepared" and does not include naturally occurring sugars such as the lactose in milk.[3] Similarly, the WHO recommends that adults and children limit intake of added sugars to 10% or less of total daily caloric intake and that further reductions to below 5% of daily intake "would provide additional benefits."[266,267] These added sugar policies present a unique challenge for fermented dairy products, such as yogurt in which the bacteria used during fermentation consume naturally occurring sugars in milk. These products tend to be tart and are often sweetened to enhance palatability. While noncaloric sweeteners may similarly enhance palatability without contributing "added sugars," the 2015 DGAC specifies that sugar substitutes should also not be relied upon because little evidence exists of their long-term effects.[97] These guidelines suggest that dairy foods low in both added sugars and in sugar substitutes are better choices for health.

The formulation of dairy products that are higher in fat may be one way for the food industry to keep dairy products palatable as well as health-promoting. Although the 2010 DGA and 2015 DGAC recommend against intakes of saturated fat greater than 10% of total caloric intake, recent evidence suggests that dairy fat may not pose the same health risks as the saturated fatty acids in other foods and may actually confer health benefits. High-fat dairy food consumption has negative associations with bone health[188,190] and cognitive health[250] but positive associations with central adiposity[207,208] and diastolic blood pressure in men.[225] Although the 2010 DGA recommend that children over the age of 2 consume low-fat or fat-free dairy products,[93] some of the studies in this review as well as other reviews and a meta-analysis[268–270] suggest that dairy fat may

actually have health benefits. One recent review even suggests that the structure of milk fat may have unique health-promoting properties.[268] Specific fatty acids in dairy foods may also promote health.[235,239,242] Increasing the fat content of dairy products may also be a way to mitigate the difficulties of reducing sugar in dairy products and retain flavor, texture, and overall palatability.

Additionally, recent consumer surveys suggest that consumers prefer "natural sweeteners," such as honey, maple syrup, and concentrated fruit juice, over sucrose or high-fructose corn syrup.[271] Full-fat dairy products with small amounts of natural sweeteners may be one way for the food industry to promote the health of its consumers in a way that aligns with both emerging scientific evidence and consumer preference.

Protein

The Institute of Food Technologists suggests in a recent publication that half of consumers are "trying to get more protein."[271] Adequate protein intake is necessary for everyone, and protein intake beyond the 0.8 g/kg may be beneficial for older adults, athletes, and children.[180,200,272] Although dairy foods contain highly bioavailable protein, because they are not included in the "protein group" of the MyPlate visual food guide,[3] consumers may not be aware of their protein content. Educating consumers about the high quality of protein in dairy foods may be an additional way to encourage individuals to consume the recommended amounts of dairy products and promote public health.

Cultured dairy products

Finally, a recent publication asserts that consumers are more receptive to "traditional foods" and products made via traditional procedures instead of products made with technologically advanced production methods or "atypical raw materials."[273] One example of this phenomenon in the American market is the popularity of skyr, an Icelandic-style yogurt. The popularity of this product, which is relatively new to U.S. consumers but a "traditional" fermented dairy product, suggests that other unfamiliar fermented products made with familiar ingredients may experience similar success.

Additionally, because yogurt and yogurt products are also considered top food trends,[271] the use of dairy cultures that consume sucrose in lieu of lactose may be an additional way to incorporate the latest nutrition policy recommendations with consumer preferences. Although products made with primarily sucrose-consuming cultures would likely not fit the FDA's standard of identity for yogurt,[263] a cultured dairy food with low added sugars that retains its naturally occurring sugars could be palatable, health-promoting, and in-line with current recommendations.

Conclusions

Dairy products have promise as health-promoting foods for the prevention or amelioration of osteoporosis, sarcopenia, the metabolic syndrome, cardiovascular disease, cognitive decline, and digestive ailments. Dietary guidelines from many countries already

recognize the importance of dairy foods. Given their unique nutrient profile as well as the ubiquity of chronic health problems around the world, with further nutrition science research and thoughtful formulations from the food industry, dairy and dairy-derived components could be used to great advantage to support public health.

Table 3-1: Contribution of the nutrients in 1 serving of milk to daily requirements of adolescent females in the U.S.

Bone health nutrient present in dairy	**Recommended daily intake**[***176]	**Amount present in 8 oz Milk**[6†††]
Calcium	1,300 mg	293 mg
Lactose	n/a	12 g
Magnesium	240 mg (ages 9-13) 360 mg (ages 14-18)	27 mg
Phosphorus	1,250 mg	224 mg
Potassium	4.5 g (ages 9-13) 4.7 g (ages 14-18)	342 mg
Protein	34 g (ages 9-13) 46 g (ages 14-18)	8 g
Vitamin D	600 IU	120 IU
Zinc	8 mg (ages 9-13) 9 mg (ages 14-18)	1.17 mg

[***] Recommended intake here listed for females ages 9-18

[†††] From USDA National Nutrient Database for Standard Reference (milk, reduced fat, 2% milk fat, with added vitamin A and vitamin D)

Table 3-2: Most common risk factors for metabolic syndrome, from the NCEP ATP III[205]

Common metabolic syndrome risk factors	Defining level
Central obesity (waist circumference)	Varies, see Table 3-3
Blood pressure	≥130/85 mmHg
Fasting glucose	≥110 mg/dL

Table 3-3: Threshold for waist circumference to indicate central obesity by population, from a Joint Scientific Statement of the International Diabetes Federation (IDF) Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity[206]

Population	**Organization**	**Waist circumference defining level for central obesity**	
		Men	*Women*
Asian	IDF, World Health Organization	≥90 cm	≥80 cm
Ethnic Central and South American	IDF	≥90 cm	≥80 cm
European	IDF	≥94 cm	≥80 cm
Middle Eastern	IDF	≥94 cm	≥80 cm
Sub-Saharan African	IDF	≥94 cm	≥80 cm
United States	National Cholesterol Education Program	≥102 cm	≥88 cm

Table 3-4. Health claims applicable for select dairy products in the United States and the European Union

Organization	Health claims	Sample applicable dairy products
FDA	Calcium, vitamin D, and osteoporosis[263]	Milk (2%, 1%, fat-free)[264] Plain yogurt (low-fat, fat-free)[264]
FDA	Sodium and hypertension[263]	Milk (2%, 1%, fat-free)[264]
FDA	Dietary lipids and cancer[263]	Milk (1%, fat-free)[264] Plain yogurt (low-fat, fat-free)[264] Cottage cheese (1%, fat-free)[264]
FDA	Dietary saturated fat and cholesterol and risk of coronary heart disease[263]	Milk (fat-free)[264] Plain yogurt (fat-free)[264] Cottage cheese (fat-free)[264]
FDA	Potassium and the risk of high blood pressure and stroke[263]	Milk (fat-free)[264]

EFSA‡‡‡	Calcium contributes to normal blood clotting[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Calcium and normal energy-yielding metabolism [274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Calcium contributes to	Milk (2%, 1%, fat-free)

‡‡‡ All calculations for applicable dairy products meeting EFSA health claim standards were completed using the USDA National Nutrient Database for Standard Reference. The following specific products were used:

- Milk, fluid 2% with added vitamin A and vitamin D
- Milk, fluid 1% with added vitamin A and vitamin D
- Milk, nonfat, fluid, with added vitamin A and vitamin D (fat-free or skim)
- Yogurt, plain, low-fat, 12 grams protein per 8 ounce
- Yogurt, plain, skim milk, 13 grams protein per 8 ounce
- Cheese, cottage, low-fat, 2% milk fat
- Cheese, cottage, low-fat, 1% milk fat
- Cheese, cottage, nonfat, uncreamed, dry, large or small curd

Vitamin D amounts used in the above calculations were the values for D2+D3 (listed in µg) and for vitamin D the values used were the RAE values (also listed in µg).

	normal muscle function[274]	Yogurt (low-fat, fat-free)
EFSA	Calcium contributes to normal neurotransmission[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Calcium contributes to the normal function of digestive enzymes[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Calcium has a role in the processes of cell division and specialization[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Calcium is needed for the maintenance of normal bones[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Calcium is needed for the maintenance of normal teeth[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)
EFSA	Reducing consumption of saturated fat contributes to the maintenance of normal blood cholesterol levels[274]	Milk (fat-free) Yogurt (fat-free) Cottage cheese (fat-free)
EFSA	Reducing consumption of sodium contributes to the	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)

	maintenance of normal blood pressure[274]	
EFSA	Live cultures in yogurt or fermented milk improve lactose digestion of the product in individuals who have difficulty digesting lactose[274]	May be applicable to some yogurt and fermented milk products
EFSA	Phosphorus contributes to normal energy-yielding metabolism[274]	Yogurt (low-fat, fat-free) Cottage cheese (2%, 1%, fat-free)
EFSA	Phosphorus contributes to normal function of cell membranes[274]	Yogurt (low-fat, fat-free) Cottage cheese (2%, 1%, fat-free)
EFSA	Phosphorus contributes to the maintenance of normal bones[274]	Yogurt (low-fat, fat-free) Cottage cheese (2%, 1%, fat-free)
EFSA	Phosphorus contributes to the maintenance of normal teeth[274]	Yogurt (low-fat, fat-free) Cottage cheese (2%, 1%, fat-free)
EFSA	Protein contributes to the maintenance of muscle	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free)

	mass[274]	Cottage cheese (2%, 1%, fat-free)
EFSA	Protein contributes to the maintenance of normal bones[274]	Milk (2%, 1%, fat-free) Yogurt (low-fat, fat-free) Cottage cheese (2%, 1%, fat-free)
EFSA	Vitamin D contributes to normal absorption/utilization of calcium and phosphorus[274]	Milk (2%, 1%, fat-free)[§§§]
EFSA	Vitamin D contributes to normal blood calcium levels[274]	Milk (2%, 1%, fat-free)
EFSA	Vitamin D contributes to the maintenance of normal bones[274]	Milk (2%, 1%, fat-free)
EFSA	Vitamin D contributes to the maintenance of normal muscle function[274]	Milk (2%, 1%, fat-free)
EFSA	Vitamin D contributes to the maintenance of normal teeth[274]	Milk (2%, 1%, fat-free)
EFSA	Vitamin D contributes to	Milk (2%, 1%, fat-free)

[§§§] EFSA vitamin D claims apply to vitamin D- fortified milk

	the normal function of the immune system[274]	
EFSA	Vitamin D has a role in the process of cell division[274]	Milk (2%, 1%, fat-free)

Table 3-5. Nutrient components in a glass of milk and their respective biological activities or health benefits

Dairy component	**Amount in 1 cup of milk (244 g)**[****]	**Proposed biological activity or health benefit**
Macronutrients		
Carbohydrate	11.71 g[6]	
Galactooligosaccharides	n/a	Proliferation of beneficial gut microorganisms, prebiotic
Lactose	12.32 g[6]	Calcium absorption
Fat	7.93 g[6]	
Conjugated linoleic acid	Organic: 0.06 g[214]	Fat mass reductions
	Conventional: 0.05 g[214]	
Heptadecanoic acid	0.4% (by weight)[238]	Contributes to insulin sensitivity
Lauric acid	3.3% (by weight)[238]	Increased HDL-C levels
Milk fat globule membrane	n/a	Cognitive health benefits, delayed onset of Alzheimer's disease
Myristic acid	10.9% (by weight)[238]	No effect on total cholesterol to HDL-C ratio

[****] Whole milk (3.25% milk fat) with added Vitamin D

Oleic acid	22.8% (by weight)[238]	Improved HDL-C and LDL-C levels
Pentadecanoic acid	0.9% (by weight)[238]	Contributes to insulin sensitivity
Trans-palmitoleic acid	0.03 g[214]	Associated with lower incidence of diabetes and insulin resistance
Protein	7.69 g[6]	Bone health, prevention of sarcopenia
Casein-derived lactotripeptides	n/a	ACE inhibitors, cognitive health benefits
Whey-derived bioactive peptides	β-lactoglobulin: 0.47- 0.95 g[275]	ACE inhibitors, cognitive health benefits
	α-lactalbumin: 0.24- 0.36 g[275]	
Micronutrients		
Calcium	276 mg[6]	Bone mass density, blood pressure regulation, decreased risk of central adiposity
Magnesium	24 mg[6]	Calcium homeostasis, prevention of bone resorption
Phosphorus	205 mg[6]	Bone mass density, bioavailability of calcium
Potassium	322 mg[6]	Bone mass density, blood

		pressure regulation
Vitamin D (fortified)	124 IU[6]	Calcium uptake, blood pressure regulation
Zinc	0.90 mg[6]	Bone formation, bone health

Table 3-6. A selection of dietary guidelines from different countries that include dairy foods

Country or Region	Dairy consumption recommendation
Australia	The Australian Dietary Guidelines recommend "at least 2 servings of reduced fat milk, yogurt, cheese or alternatives every day." A serving is defined as 1 cup milk, a small container of yogurt, or 1 slice of cheese[94]
Canada	Canada's Food Guide recommends 2 daily servings of "milk and alternatives" for adults and suggests the following options: lower fat milk, canned milk, fortified soy beverage, yogurt, kefir, and cheese[95]
England	England's EatWell Plate suggests consuming "some milk and dairy foods" daily including lower-fat milk and lower-fat yogurt but recommends limited consumption of most cheeses[276]
France	Le Guide Alimentaire Pour Tous recommends consuming 3 daily servings of dairy products such as milk, yogurt, cheese, and cottage cheese but limiting consumption of dairy-based desserts[134]
Greece	This guide recommends 2 daily servings of dairy products, especially fat-free products[135]
Greenland	Greenland's food-based dietary guidelines do not include a

	specific recommendation to consume dairy products in their 10-item list but does state that consumers should select lower-fat milk and cheese[96]
Hungary	Three to 4 servings of milk and dairy products (example: 1 glass of milk, kefir, or yogurt, 50 g cottage cheese, or 30 g cheese) should be consumed each day[277]
Japan	Japan's Food Guide Spinning Top recommends 2 daily servings of milk and milk products and defines ½ cup of milk as 1 serving[278]
Oman	The Omani Guide to Healthy Eating suggests 1 daily serving, such as 1 cup of milk, 1 cup of yogurt, or 45 g cheese, from the "milk and dairy" group[92]
Singapore	Singapore's My Healthy Plate guide recommends 2-3 daily servings from the "meat and others" group, which includes milk and cheese. Two glasses of milk or 2 slices of cheese are listed as sample servings[279]
Sweden	Swedish Nutrition Recommendations state that low-fat, unsweetened dairy products with vitamin D are best and adults need 2-5 deciliters a day[137]
Switzerland	The Swiss Pyramide Alimentaire recommends 3 servings of milk or milk products daily. A serving is defined as 2 deciliters of milk or 30 g of cheese[280]

United States	The 2010 Dietary Guidelines for Americans recommend that adults consume 3 cups of fat-free or low-fat milk and milk products daily[93]

Section 2: Mushrooms

Chapter 4: Impact of *Agaricus bisporus* mushroom consumption on satiety and food intake

The original version of this article can be found in Appetite 117(2017);179-85. This article was co-authored by Qi Wang, Clarissa Kraft, and Joanne Slavin. The reprint of this article has been used with permission from the publisher, as published in agreed format.

Summary

Previous studies on mushrooms suggest that they can be more satiating than meat, but this effect has not been studied with protein-matched amounts. The objective of this study was to assess the differences with satiety and ten-day food intake between *A. bisporus* mushrooms (226 g) and meat (28 g) in a randomized open-label crossover study. Thirty-two healthy participants (17 women, 15 men) consumed two servings of mushrooms or meat for ten days. On the first day, fasted participants consumed protein-matched breakfasts. Participants rated their satiety using visual analogue scales (VAS) at baseline and at regular intervals after the meal. Three hours later, participants were served an *ad libitum* lunch. Participants were given mushrooms or meat to consume at home for the following nine days. Energy intake was assessed at the *ad libitum* lunch, and participants also completed diet diaries on the day of the study, day 2, and day 10. Participants reported less hunger ($p=0.03$), greater fullness ($p=0.03$), and decreased prospective consumption ($p=0.02$) after the mushroom breakfast. There were no

significant differences in participant ratings of satisfaction (p=0.06). There were also no differences in energy intake at the *ad libitum* lunch or with the diet diaries from days 1 (p=0.61), 2 (p=0.77), or 10 (p=0.69). Mushroom consumption did increase fiber intake on days 1 (p= 0.04) and 2 (p= 0.0001) but not on day 10 (p=0.29). The mushroom intervention also did not affect energy intake over the 10-day feeding period.

Introduction

Rising rates of obesity,[281] which currently affects 34% of U.S. adults, has made understanding influences on satiety and food intake urgent. Satiety is the postprandial state responsible for the timing and intake of the next meal.[12,13,282] Increasing scientific understanding of satiety is of great importance for both clinical treatment of obesity and public health prevention efforts. Consuming satiating meals that promote a feeling of fullness could result in decreased daily caloric intake and, over time, assist with weight loss and weight management.[35,283] A great deal of research has been conducted on the satiating abilities of different macronutrients.[13,91,284–288] Protein appears to be more satiating than either carbohydrates or fat.[284,285,287] Yet not all carbohydrates exert the same influence on satiety.[286] Fiber-rich foods, for instance, tend to be more satiating than foods high in sugars and starches.[288] The type and form of fiber in whole foods versus isolated fiber sources impacts its satiating effects.[13,289] While many studies have been conducted on isolated fiber types,[26,290–297] less is understood about the satiety effects of fibers served in whole foods.[13,19,30]

A few previous studies have addressed the impact of white button mushroom

consumption on satiety and food intake.[31,32] Cheskin et al. compared the impact of mushroom or meat-based lunches on satiety and energy intake in 76 individuals.[31] There were no significant difference in satiety ratings between the meat and mushroom lunches. However, because this study matched the lunch meal interventions by volume, a lower number of calories from mushrooms (339 kcal) was as satiating as a higher number of calories (783 kcal) from meat. A second study[32] conducted by the same research team found that replacing meat with mushrooms at three meals a week for one year increased the amount of weight lost over six months, helped participants maintain their weight loss for six months, and led to decreased body mass index (BMI) and waist circumferences. The results of these studies suggest that mushrooms enhance satiety and that substituting white button mushrooms for meat may decrease the energy density (kcal/g) of the diet, resulting in weight loss.[298,299]

These results from mushrooms may also be due to their macronutrient composition. Mushrooms contain both protein and fiber.[6,7,300] While white button mushrooms contain a relatively small amount of protein (3.09 g/100 g),[6] mushroom protein is of moderate quality.[4,300] Mushroom protein has protein quality rating, or protein digestibility corrected amino acid score (PDCAAS), of 0.66.[9] PDCAAS scoring evaluates protein quality based on limiting amino acids, fecal digestibility, and the protein needs of preschool-aged children, with higher values given to higher quality proteins.[24] The highest quality protein sources in this index are animal sources, such as milk and eggs (PDCAAS value of 1.00), while wheat protein has a PDCAAS value of 0.42.[24] Cooked lentils have a PDCAAS value of 0.66, like mushrooms.[301] Mushrooms have a protein

quality rating higher than grains but comparable to other non-animal protein sources.[24,301] Mushrooms also contain several different types of non-digestible carbohydrates including chitin, β-glucans, raffinose, oligosaccharides, and resistant starch.[6,7,300]

To build on the results of previous studies on white button mushrooms, we designed a study to assess the satiety response and food intake of 32 participants after consuming protein-matched amounts of mushrooms and meat in a randomized crossover study. Participants consumed test foods at breakfast and at dinner for a total of ten days. On the first day of each intervention, participants visited the lab to consume a mushroom or meat-based breakfast sandwich. Following this meal, we measured energy intake at an *ad libitum* lunch and for forty-eight hours following the test visit. We also assessed dietary intake for twenty-four hours prior to the test visit and after nine days of test food consumption. Our hypothesis was that the mushroom intervention would provoke a greater satiety response than the control (meat) meal and that the treatment diet would result in a lower average energy intake (kcals/day) than the control diet. Unless indicated otherwise, in the remainder of this manuscript, the word "mushrooms" indicates "white button mushrooms" or white, immature *Agaricus bisporus*.

Materials and Methods

In this randomized crossover study, we compared the impact of mushroom consumption and meat consumption on satiety and ten-day food intake. Participants were provided with test foods to consume for ten days, beginning with an in-lab test meal containing either mushrooms or meat.

Subjects

The University of Minnesota Institutional Review Board Human Subjects Committee reviewed and approved all methods for human participants, and all participants provided written informed consent. Participants were recruited by flyers placed around the University of Minnesota campus and were asked to complete an online screening survey (Qualtrics, Provo, UT). Eligible subjects included healthy men and women between the ages of 18 and 65 with a body mass index between 18.5 and 30 kg/m^2. Subjects had to be regular breakfast and lunch consumers (≥4 times per week) willing to consume meat and mushrooms. Participant demographics are listed in Table 4-1.

Excluded individuals included people with serious preexisting health conditions (diabetes, kidney/liver disease, cancer, eating disorder) and individuals taking medications for blood sugar, cholesterol, blood pressure, or weight loss as well as individuals taking laxatives or anti-diarrhea medications. Individuals who had gained or lost more than 10 pounds in the last three months, were regular participants in vigorous endurance exercise (marathons, endurance bike races, triathlons), or were tobacco users were also excluded. In addition, individuals could not have participated in another dietary intervention study within the last month, had to be willing to make dietary changes for a total of 20 days, and could not have food allergies. Pregnant or lactating females were excluded. Participants could not be regular fiber consumers (had to consume ≤3 servings of fiber-rich foods per day) and could not take supplements besides a multivitamin.

Individuals with a score >11 on the dietary restraint portion of the Three Factor Eating Questionnaire were also disqualified. Participants had to be available to attend two in-person visits on weekend mornings from 7:45am to 11:30am.

Before arriving for the first in-person study visit, participants made an initial study visit to review the informed consent paperwork and provide their height and weight measurements. Height was self-reported by study participants, and weight was measured by the same lab technician using the same digital scale for all participants.

Thirty-five participants completed the informed consent process. Two female participants dropped out of the study before attending any sessions due to scheduling conflicts, and one male participant dropped out of the study halfway through due to dislike of mushrooms. Thirty-two participants (17 women, 15 men) completed the entire study.

Participants were asked not to consume beef during the mushroom feeding intervention of the study and to avoid mushrooms during the meat intervention of the study. Participants were also instructed to maintain their normal activity level and refrain from consuming laxatives as well as any pre or probiotic foods. Participants had constant access to study staff to ask any questions or report any concerns.

In-person Study Visits

Participants were asked to refrain from consuming alcohol or exercising heavily (beyond a normal routine) for twenty-four hours prior to each study visit. Participants were also asked to fast for at least 12 hours prior to arriving at the test site. All visits were

held on weekend mornings. Participants arrived by 7:45am and were seated in quiet testing room where they remained for the duration of their visit, approximately 3.5 hours. As much as possible, participants were seated in the same places in the same rooms for both study visits. All lab sessions were held in the same rooms and participants were assigned seats.

Upon arriving, participants completed visual analogue scale (VAS) ratings for their hunger, satisfaction, fullness, and prospective food intake. At 8:00 am, participants were given a breakfast meal and asked to consume it within 15 minutes. Breakfast meals were sandwiches containing either mushrooms roasted with olive oil or sautéed 93% lean ground beef cooked in olive oil along with an English muffin (Thomas®), one baked egg, and two cheese slices (Market Pantry™ Mild Cheddar Deli Sliced Cheese). Sandwiches were matched for protein content and had similar energy contents (Table 4-2). Due to the difference in volume between the meat and mushrooms, neither participants nor the research team were blinded to the treatments provided at each study visit.

Participants were offered their choice of 6 fl oz of black coffee or tea to accompany the breakfast sandwich as well as a 12 fl oz bottle of water. Participants were asked to consume the entire sandwich but did not have to drink all of the coffee or tea. Participants were allowed to continue drinking the bottle of water provided throughout the duration of the study and could request additional bottles for consumption *ad libitum* throughout the study.

Participants completed additional VAS measures 15, 30, 45, 60, 90, and 120 minutes after baseline. At 180 minutes after baseline, participants were given an *ad*

libitum pizza meal. Each participant was given approximately 15 minutes to eat until they felt comfortably full.

Ten-Day Feeding Intervention

When leaving the in-person study visits, participants were given either mushrooms or ground beef to continue eating for a total of ten days, twice a day. Mushrooms provided were sliced mushrooms (Giorgio) and beef provided was 93% lean, 7% fat (Market Pantry). These foods were matched for protein content and had similar energy contents (Table 4-3).

Mushrooms provided were raw and packaged in individual serving containers (226 g). Participants were asked to consume two containers, a total of 452 g of mushrooms, daily. Due to the short shelf life of mushrooms, participants consuming the mushroom test food were provided with 7 servings of mushrooms (enough for the first four days of the study) and had to return to the lab to pick up the remaining 12 mushroom servings on day 4 or day 5 of the intervention. Participants completing the mushroom arm of the study were given a handout with suggested cooking methods for the mushrooms. Participants were encouraged to cook the mushrooms prior to eating.

Ground beef was provided pre-cooked (by the research team) and frozen in individual 28 g servings. Participants were asked to consume 56 g of study-provided meat daily. Participants were provided with all 19 servings of meat on the day of their test visit and instructed to keep the meat frozen until consumption.

Participants had a minimum ten-day washout period between treatments.

Outcome measures

VAS

A shortened version of a validated VAS questionnaire was used to assess satiety response.[302] For these questionnaires, participants responded to prompts to indicate their levels of satiety, hunger, fullness, and prospective food consumption. To indicate their status, participants drew a mark on a 10 cm line. The four questions were: "How hungry do you feel?" I have never been more hungry (0 cm) → I am not hungry at all (10 cm), "How satisfied do you feel?" I am completely empty (0 cm) → I cannot eat another bite (10 cm), "How full do you feel?" Not at all full (0 cm) → Totally full (10 cm), "How much do you think you can eat?" A lot (0 cm) → Nothing at all (10 cm). VAS questionnaires were provided as hard copies and included in a folder of study documents provided to each participant at each study visit.

Food Intake

Participants were instructed to record all meals, snacks, beverages, and condiments consumed for twenty-four hours prior to each study visit (beginning 8:00 am the day before in-person sessions). Participants also recorded all foods and beverages consumed after leaving the lab following each in-person visit. Participants were additionally asked to record all food and beverage consumption on the second day of eating each study food

and on the tenth day of eating each study food. Participants were provided with a printed sheet listing portion guidelines and examples. These records allow us to compare average nutrient intakes of participants eating the meat versus mushroom study foods.

Ad libitum meal consumption

At 180 minutes after baseline, participants were each provided with one Jack's Original Cheese pizza (960 kcal total) cut into 12 different sized wedges. Participants were given approximately 15 minutes to eat until they felt comfortably full. After 10 minutes, if participants had finished eating the entire pizza, they were asked if they would like more pizza. If participants finished one pizza, they were provided with a second entire pizza. Pizzas were weighed before and after consumption to determine calories consumed.

Compliance

Participants were given a checklist to track on which days they ate and did not eat the study foods and were also asked to turn in 24-hour food diaries for days 1 (in-person visit), 2, and 10.

Data Analysis and Statistical Methods

Data from VAS was assessed by first calculating the area under the curve (AUC) from baseline to 120 minutes using the trapezoidal rule. These values were then corrected

for the baseline values (mean baseline adjusted AUC). Finally, the differences between the baseline-corrected AUC were evaluated with paired t-tests.

Diet records were analyzed using the Nutrition Data System for Research (NDSR) software version 2015 developed by the Nutrition Coordinating Center at the University of Minnesota, Minneapolis, MN. This software includes nutrition information on several branded and non-branded food products. Diet records were analyzed for total energy (kcal), fat, protein, carbohydrate, and fiber consumption.

A sample size of 32 gave us 81% power to identify as significant a mean difference of 0.7 SD in the VAS scores between the two diets (mushroom and meat). Participants stratified by sex were randomly assigned to the two sequence groups (mushroom-meat and meat-mushroom). Participants' demographics and characteristics were summarized and compared between the two sequence groups using Chi-square tests and two-sample t tests for categorical and continuous variables respectively. The effects of mushroom consumption and meat consumption on outcome measures were evaluated using paired t-tests. Statistical analyses were performed using the Statistical Analysis System (SAS, version 9.3, 2011; SAS Institute, Cary, NC). Two-sided tests with p-value less than 0.05 were considered statistically significant.

Results

VAS

Most of the VAS results showed a significant difference between the mushroom

and meat treatments. Participants reported significantly less hunger (p=0.03), greater fullness (p=0.03), and decreased prospective consumption (p=0.02) after the mushroom treatment in comparison to the meat treatment. There were no significant differences in baseline-corrected area under the curve for participant ratings of satisfaction, however (p=0.06) (Table 4-4).

Food Intake

Ad libitum Meal Consumption

While participants ate more calories of the pizza lunch after consuming the meat-based breakfast treatment (740.63 ± 274.17) than after consuming the mushroom-based breakfast treatment (684 ± 202.87), the difference between the total calories consumed after both preload meals was not statistically significant (p=0.06) (Table 4-4).

Diet Diary Food Intake

The day before the in-person study visits (Day 0), there were no significant differences between the groups in terms of energy, fat, protein, carbohydrate, or fiber consumed (Table 4-5). After the intervention on Day 1, however, diet records from participants after leaving the study site (i.e. diet records that do not include the breakfast intervention or the *ad libitum* meal consumption information) show that participants consumed significantly more fiber on the first day of the mushroom diet (p=0.04) (Table 4-6). On Day 1, participants did not consume significantly different amounts of energy,

fat, protein, or carbohydrates. Participants also consumed significantly more fiber on the mushroom diet on the second day of the study (p=0.0001) (Table 4-7). However, on day 10, there were no significant differences in energy, total fat, protein, carbohydrate, or fiber consumed by participants (Table 4-8).

Compliance

For the mushroom diet, 20 participants turned in fully completed checklists. Five participants turned in mostly complete checklists with five or fewer missed servings. Seven participants turned in blank checklists or were missing checklists altogether. For the meat diet, 22 participants turned in fully complete checklists, four turned in partially completed checklists, and six participants had missing or blank checklists.

Discussion

The results of this study show that mushrooms had a greater impact on VAS ratings of hunger, fullness, and prospective consumption than a meat control and that adding mushrooms to the diet significantly increased the fiber intake of low-fiber adult consumers over a short-term period. However, a mushroom preload meal did not affect energy intake at an *ad libitum* lunch meal, and a ten-day long mushroom-rich diet did not impact energy, fat, carbohydrate, or protein intake compared to a control diet.

The mushroom and meat-based breakfast meals were matched for protein content but not for fiber, carbohydrate, fat, energy content, or total weight. The differences in

portion size and fiber content between the two sandwiches may explain the greater satiating effect of the mushroom sandwich. The strongest predictor for satiety may be portion size, especially for overweight and obese individuals, who need to consume greater volumes of food to feel satiated.[298,303] In our study, the mushroom sandwiches were larger than the meat ones, because the volume of mushrooms needed to match the protein in a small amount of beef was quite large (about 105 g cooked of mushrooms versus 28 g of beef cooked). Mushrooms also contain more water than meat, which also contributes to their volume.[13] This greater volume of mushrooms would likely take more time and effort to chew. Chewing promotes saliva and gastric acid secretion, both of which may increase gastric distention and promote a feeling of fullness.[13,35]

Fiber content, which also contributes to food volume, may also contribute to the greater satiating effect of the mushroom breakfast.[12,13] Like the act of chewing, fiber consumption may also increase gastric distention and slow gastric emptying, promoting fullness and satiation, depending on the type of fiber present.[13,23,304] With whole foods like mushrooms, it is difficult to identify which fiber(s) present may be responsible for the effect on satiety. Mushrooms contain several different types of fiber, including both fermentable and non-fermentable types.[7,300] While the impact on satiety of some of the fibers in mushrooms, like resistant starch and mycoprotein (which contains a fibrous chitin and β-glucan matrix), have been studied as isolated sources,[291,293,296] it is difficult to predict how these fibers, much less a heterogeneous combination of fibers, will interact in a food matrix. Furthermore, though fiber is often considered to have a positive correlation with satiety, satiety studies assessing the impact of fiber in both isolated[290,292–

[295,297] and whole foods forms[19,20] have had mixed results. While the fiber content from the breakfast meal may have influenced VAS ratings of satiety, our results do not suggest evidence for a uniquely satiating effect of mushroom fibers.

While participants reported feeling more satiated after the mushroom breakfast, they did not adjust their energy intake at the *ad libitum* lunch. The portion size and fiber content (4 g) of the mushroom breakfast meal might have made participants feel fuller initially but was not sufficiently different from the meat meal, which contained 1 g of fiber, to impact energy intake at the next meal. Previous research suggests that fiber intake may need to be increased by about 14 g per day for at least two days to decrease energy intake by 10%.[304]

Similarly, the 4.6 g of fiber provided daily throughout the ten days of the mushroom intervention significantly increased fiber consumption on days 1 and 2 but did not affect energy intake. Participants were recruited for this study based in part on their consumption of a "typical" amount of fiber for American consumers, approximately 15 g.[97] The amount of fiber participants consumed throughout the study averaged between 10 and 22 g daily, still below the recommended 25 g daily for women and 38 g daily for men.[44] Nevertheless, increasing fiber is widely regarded as beneficial for health, even if it does not affect energy intake or satiety.[97,305] People who eat higher amounts of fiber tend to have lower body weights than people who eat less fiber,[305] and fiber is one of four underconsumed nutrients of public health concern in the 2015-2020 Dietary Guidelines for Americans.[44] In addition to the changes in fiber intake during the mushroom arm of this study, fiber intake varied greatly during the beef arm of this study as well, from about

10 g on Day 1 to over 20 g on Day 10. While the mushroom treatment had a significant impact on fiber consumption on day 2, the variation in fiber intake throughout the study during both the mushroom and meat arms suggests that day to day fiber intake may fluctuate greatly regardless of dietary intervention.

While the large amount of mushrooms participants consumed in this study was well-tolerated, this amount is much higher than average consumption,[306] and it is unlikely that people would choose to consume this amount of mushrooms daily. However, substituting mushrooms for meat a few times a week may increase fiber intake while also increasing compliance to the U.S. dietary guidance to increase protein intake from non-animal sources. Besides fiber, mushrooms also contain potassium and vitamin D (with exposure to UV light), other nutrients "of public health concern" in the U.S.[44] In addition, a recent sensory study[43] showed that substituting some of the meat in tacos reduced calories, sodium, and fat in the dish while actually increasing perceived flavor intensity, adding fiber, and decreasing the perceived amount of sodium needed. Mushrooms may not replace meat, and perhaps should not replace meat in omnivorous diets (as meat is an excellent source of iron and bioavailable protein), but adding mushrooms to meat may be a practical avenue to supplement meat with non-animal proteins[44] without necessitating a decrease in portion size or requiring fully vegetarian meals.

There are some limitations to our findings, however. While we utilized a crossover design, which strengthened the study, our study design was "open-label," so both participants and research team were aware of which treatment participants were receiving. This study design means there is a possibility of bias affecting the

interpretation of our results. An additional major limitation is the missing VAS data from 180 minutes. Due to a typo on the hard copy printouts of our VAS scales, participants received incorrectly labeled forms and did not complete a VAS at 180 minutes, which would have been a valuable addition to the satiety data discussed in this study.

In addition, several of our outcome measures relied on self-report. We also relied on participants to self-report their heights. A systematic review indicated that participants tend to overestimate their heights.[307] If the participants in this study overestimated their heights, it is possible that our population actually included several more overweight or obese individuals. This change would affect our results, as research indicates that overweight and obese participants tend to underreport food intake on diet diaries.[308–310]

Compliance to both the mushroom and meat dietary interventions was also based on self-report but almost a third of the study participants turned in partially completed or blank checklists. However, at the in-person visit, all participants consumed the breakfast sandwich, and no adverse symptoms were reported. While the daily quantity of mushrooms participants were asked to consume was quite large (equivalent to over five servings),[311] only one participant complained about the large quantity of mushrooms. Finally, there was a slight difference in the energy content of the mushroom breakfast compared to the meat breakfast as well as a difference in fat and carbohydrate content, which limits the accuracy of our satiety data.[282]

Conclusions

The results of this study build on results of previous studies showing a greater

impact on satiety from mushrooms compared to meat. Our results show an impact on satiety when mushrooms and meat meals are matched for protein content, though this impact did not affect energy intake. Adding mushrooms to the diet for ten days created a trend for higher fiber intake, an underconsumed nutrient of concern, in this study. There may be a benefit to consumers to substitute mushrooms for meat in some meals or replace some of the meat content of meals with mushrooms to increase vegetable and fiber intake as well as protein from sustainable non-animal sources.

Table 4-1. Participant demographics overall and by treatment group

	Overall (N=32)	**Mushroom-Meat (N=16)**	**Meat-Mushroom (N=16)**	**P-value**
Sex, N (%)				
F	17 (53%)	7 (44%)	10 (62%)	0.29
M	15 (47%)	9 (56%)	6 (38%)	Chi-Square statistics (df=1) = 1.13
Age, mean (SD)	23.4 (4.4)	23.6 (4.8)	23.3 (4.0)	0.81 t (df=30) = 0.24
BMI (kg/m^2), mean (SD)	24.2 (3.2)	25.3 (3.1)	23.0 (2.8)	0.03 t (df=30) = 2.27

Table 4-2. Nutrition composition of mushroom and meat breakfast meals at in-person study visit

Treatment type	Kcal	Total fat (g)	Carbohydrates (g)	Fiber (g)	Protein (g)
Mushroom Sandwich	514	45.5	31	4	26
Meat Sandwich	507	47.5	25	1	26

Table 4-3. Nutrition composition of each serving of mushrooms and meat provided for ten day food intake assessment

Treatment type	Kcal	Total fat (g)	Carbohydrates (g)	Fiber (g)	Protein (g)
Mushroom (226 g)	50	0	6	3	6
Beef (28 g)	43	2	0	0	6

Table 4-4. Results for VAS ratings of satiety and energy intake at *ad libitum* lunch

Visual Analogue Scales††††	**Mushrooms (n=32)**	**Meat (n=32)**	**P-value**
Hunger (cm/min)	1038.7 ± 59.6	888.6 ± 57.5	0.045 t (df=31) = 2.09
Satisfaction (cm/min)	1061.8 ± 48.8	963.2 ± 51	0.10 t (df=31) = 1.69
Fullness (cm/min)	1129.9 ± 57.1	981.4 ± 60.1	0.05 t (df=31) = 2.02
Prospective consumption (cm/min)	996 ± 52.3	879.1 ± 52.9	0.03 t (df=31) = 2.29
Pizza lunch (kcal)‡‡‡‡	684 ± 52	740 ± 71	0.06 t (df=31) = -1.94

†††† Data presented as the mean baseline corrected area under the curve ± SEM.

‡‡‡‡ Data are presented as the mean calories ± SEM.

Table 4-5. Diet diary record day 0

Macronutrient	**Mushrooms n=28**	**Meat n=28**	**P-value**
Energy (kcal)[a]	1946.00 ± 561.53	1992.89 ± 647.63	0.76 t (df=27) = -0.31
Total fat (g)[a]	75.03 ± 29.53	81.46 ± 37.63	0.50 t (df=27) = -0.68
Protein (g)[a]	75.53 ± 39.36	81.10 ± 31.13	0.51 t (df=27) = -0.67
Carbohydrate (g)[a]	241.79 ± 79.63	233.93 ± 74.62	0.69 t (df=27) = 0.40
Fiber (g)[a]	16.43 ± 7.43	15.22 ± 5.81	0.40 t (df=27) = 0.85

[a]Data presented are mean values ± standard deviation.

Table 4-6. Diet diary record day 1

Macronutrient	**Mushrooms n=31**	**Meat n=31**	**P-value**
Energy (kcal)[a]	1287.35 ± 527.34	1220.13 ± 588.42	0.61 t (df=30) = 0.51
Total fat (g)[a]	49.89 ± 30.03	49.40 ± 29.63	0.95 t (df=30) = 0.07
Protein (g)[a]	47.37 ± 24.82	42.49 ± 21.79	0.38 t (df=30) = 0.88
Carbohydrate (g)[a]	153.06 ± 65.58	150.51 ± 83.01	0.87 t (df=30) = 0.17
Fiber (g)[a]	13.22 ± 6.57	10.35 ± 5.30	0.04 t (df=30) = 2.15

[a]Data presented are mean values ± standard deviation.

Table 4-7. Diet diary record day 2

Macronutrient	**Mushrooms n=32**	**Meat n=32**	**P-value**
Energy (kcal)[a]	1919.44 ± 774.90	1961.19 ± 693.12	0.77 t (df=31) = -0.30
Total fat (g)[a]	76.78 ± 32.87	81.64 ± 34.14	0.47 t (df=31) = -0.73
Protein (g)[a]	76.22 ± 36.71	81.27 ± 33.02	0.49 t (df=31) = -0.70
Carbohydrate (g)[a]	237.85 ± 108.20	229.44 ± 93.46	0.66 t (df=31) = 0.45
Fiber (g)[a]	20.83 ± 8.01	14.49 ± 7.03	0.0001 t (df=31) = 4.33

[a]Data presented are mean values ± standard deviation.

Table 4-8. Diet diary record day 10

Macronutrient	**Mushrooms n=30**	**Meat n=30**	**P-value**
Energy (kcal)[a]	1966.3 ± 805.12	2017.83 ± 557.56	0.69 t (df=29) = -0.40
Total fat (g)[a]	87.71 ± 39.41	85.38 ± 28.33	0.78 t (df=29) = 0.29
Protein (g)[a]	77.41 ± 36.08	83.71 ± 24.75	0.39 t (df=29) = -0.87
Carbohydrate (g)[a]	227.74 ± 101.4	237.05 ± 99.04	0.57 t (df=29) = -0.57
Fiber (g)[a]	22.17 ± 8.76	20.24 ± 11.21	0.29 t (df=29) = 1.07

[a]Data presented are mean values ± standard deviation.

Chapter 5: Impact of *Agaricus bisporus* mushroom consumption on gut health markers

Summary

Background: *Agaricus bisporus* mushroom consumption may impact human gut health. These mushrooms, also known as "white button mushrooms," have a unique carbohydrate profile that includes known prebiotics including resistant starch, β-glucans, and mannitol. The impact of mushroom consumption on gut health has not been studied in a human population.

Objective: The objective of this study was to assess the effect of mushroom consumption compared to a meat control on markers of gut health, including gastrointestinal tolerance, short chain and branched-chain fatty acid production, fermentation, and laxation.

Methods: A randomized open-label crossover study was conducted in healthy adults (n=32) who consumed protein-matched amounts of mushrooms or meat twice daily for ten days. Breath hydrogen and methane measures were taken on day one, and gastrointestinal tolerance was evaluated throughout each treatment. From days 6-10, participants completed a full fecal sample collection. Samples were assessed for weight, pH, consistency, and short chain and branched-chain fatty acid concentrations.

Results: There were no differences in breath hydrogen, breath methane, or in stool frequency, consistency, fecal pH, or short chain fatty acid concentrations between the two diets. One branched-chain fatty acid, isovalerate, was found in higher concentrations during the meat diet ($p=0.02$). Although both diets were well tolerated by

participants, the mushroom diet led to greater overall gastrointestinal symptoms, including gas (p=0.045 on Day 1; p=0.005 on Day 2) and flatulence (p=0.0002 on Day 1; p=0.016 on Day 2) than the meat diet on days 1 and 2. Average stool weight was also significantly higher on the mushroom diet (p=0.002).

Conclusions: While mushroom consumption did not differ significantly from meat consumption in its impact on most gut health markers assessed in this study, the increase in stool weight and presence of undigested mushrooms in stool suggest that mushroom consumption does impact laxation and further research is warranted.

Introduction

Dietary fiber and other low- and non-digestible carbohydrates are considered important nutrients for human health.[35,44,305,312] Many studies have been conducted on their benefits both when added to the diet as supplements (in isolated forms)[294,313,314] and when provided as part of a food.[19,31,32,315] Some health benefits linked with fiber consumption include a reduced the risk of cardiovascular disease,[35] enhanced satiety, reduced postprandial blood glucose, and improved laxation.[316] Recent research suggests that consumption of fiber may also benefit the gut microbiota, especially since some fibers also function as prebiotics.[35] Certain foods like bran cereal, beans and legumes, and some fruits and vegetables are considered good sources of dietary fiber[317] and, therefore, are recommended in U.S. Dietary Guidance[44] as foods to eat in order to consume adequate dietary fiber (25 g daily for women, 38 g daily for men).[316] However, some foods, including mushrooms, that do not qualify for a “good source of fiber” label

according to U.S. Food & Drug Administration (FDA) guidance,[317] still contain low- and non-digestible carbohydrates and may benefit gut health.

The carbohydrate profile of mushrooms, which includes several different types of low-digestible and non-digestible carbohydrates, including chitin, β-glucans, raffinose, oligosaccharides, and resistant starch,[5–7,318] suggests that they may improve laxation and stimulate short-chain fatty acid (SCFA) production. Even common *Agaricus bisporus* mushrooms, or white button mushrooms, have a unique carbohydrate profile that includes low-digestible carbohydrates, such as resistant starch, β-glucans, and mannitol, known to have gastrointestinal effects.[7,300,312,319,320]

These low-digestible carbohydrates have been evaluated for their effects on gastrointestinal health or function when provided in isolated forms. Resistant starch isolated specifically from mushrooms has not been evaluated for its impact on gut health, to our knowledge. Some,[321] but not all,[322] studies on resistant starch from other sources show that it has a beneficial impact on laxation markers. This effect was primarily seen with doses ≥25 g per day. Similarly, the health impact of the fungal β-glucans isolated specifically from white button mushrooms have not been evaluated to our knowledge. However, isolated fungal β-glucans from Shiitake mushrooms (*Lentinus edodes*) and Oyster mushrooms (*Pleurotus ostreatus*), both from the same taxonomic order as *Agaricus bisporus*, have had a beneficial impact on intestinal health in studies with animal models.[323] Relatively few studies have been conducted on the gastrointestinal effects of mannitol ingestion. A 2009 review describes that mannitol is well tolerated in doses up to 20 g daily but may lead to diarrheal stools at amounts higher than 40 g.[312] In

fact, the U.S. Food and Drug Administration requires a warning label ("Excess consumption may have a laxative effect") on foods that could reasonably provide 20 g or more of mannitol in one day.[324] Mannitol has also been referred to as a prebiotic, or a "substrate selectively utilized by host microorganisms to confer a health benefit,"[36] in an animal model.[325]

These components have not been evaluated for their impact on human gut health when consumed in white button mushrooms ("mushrooms"). This impact of eating mushrooms has so far only been evaluated in animal studies. Results from animal studies suggest that some carbohydrates in mushrooms function as prebiotics in a mouse model as well as in turkey poults and broiler chickens.[37] Adding 1% white button mushrooms to the purified diet of C57BL/6 mice resulted in increased gut bacterial diversity, including increases in *Bacteroidetes* and decreases in *Firmicutes* compared with control-fed mice.[37] While not model animals, turkey poults fed *A. bisporus* mushrooms added at 0, 10 or 20 g/kg feed for 70 days increased ileal *Lactobacillus* spp. counts ($p=0.000$) in both the 10 g/kg and 20 g/kg groups compared to the control group.[39] Ileal *E. coli* populations were also significantly lower ($p=0.043$) in the 20g/kg groups compared to the other two groups. In addition, cecal *Lactobacillus* spp. ($p\leq0.05$) was higher in both mushroom-supplemented groups and *Bifidobacterium* spp. was higher in the 20g/kg group ($p=0.045$). A similar experiment[326] conducted by the same research group found that adding 0, 10, or 20 g/kg of dried mushrooms in the feed of broiler chickens for 42 days increased ileal *Lactobacilli* spp. populations ($p=0.005$) in the 20g/kg group. The mushroom diet also slightly increased cecal *Lactobacilli* spp. and *Bifidobacteria* spp.

($p=0.005$) in both supplemented groups.[326] Two additional studies conducted in broiler chickens found that adding mushrooms in amounts ranging from 10 g/kg of feed to 30 g/kg of feed decreased *E. coli* levels[327,328] compared to control diets and, in one study, also significantly increased *Lactobacilli* spp.[328] The authors of each of these studies concluded that including mushrooms in the diet of these animals beneficially influenced gastrointestinal health.

Although this research in animals is promising and the impact of mushrooms on gut health in humans has been the subject of scientific speculation for several years,[10,329–333] it has not been formally evaluated in a clinical trial prior to this study to our knowledge. The objective of this study was to assess the impact of 10 days of mushroom consumption compared to meat consumption on gut health markers in healthy adults. While this manuscript does not detail the impact of mushrooms on the fecal microbiota, a topic addressed in several of the animal studies, it does address other endpoints that have been tied to bacterial fermentation of prebiotics, including short chain fatty acid concentrations[35] and laxation markers[35,313] such as stool weight, pH, and consistency. This study also collected subjective measures of gastrointestinal tolerance as well as breath hydrogen and breath methane measurements, which serve as markers of colonic fermentation by gas-producing bacteria. To our knowledge, none of these outcomes have been evaluated in a human population with mushroom feeding.

Given the health effects observed with isolated forms of the carbohydrates found in mushrooms,[323,325,334] we hypothesized that mushroom feeding would result in higher concentrations of SCFA relative to a meat control. We also hypothesized that the

mushroom treatment would be well-tolerated by participants and result in improved laxation markers, including greater fecal bulk and a higher rate of "normal" stool consistency, compared to the meat treatment.

Materials and Methods

Subjects

Participants were recruited by flyers on the University of Minnesota's St. Paul campus and asked to complete an online screening survey (Qualtrics, Provo, UT). Healthy men and women between the ages of 18 and 65 with a body mass index between 18.5 and 30 kg/m^2 were eligible. All participants also had to be regular breakfast and lunch consumers (≥4 times per week) willing to consume both meat and mushrooms. Exclusion criteria included having a serious preexisting health condition (diabetes, kidney/liver disease, cancer, eating disorder) and/or taking medication for blood sugar, cholesterol, blood pressure, or weight loss. Individuals taking laxatives or anti-diarrheal medications or individuals who had gained or lost more than 10 pounds in the last three months were also ineligible. Pregnant or lactating females were excluded. Participants could not be regular fiber consumers (had to consume ≤3 servings of fiber-rich foods per day) and could not take supplements besides a multivitamin. Participants could not have been on antibiotics within the last three months and could not have any had any gastrointestinal conditions or surgeries. Additional inclusion and exclusion criteria as well as detailed participant demographics have been published elsewhere.[318]

Thirty-five participants completed the informed consent process. Two female participants dropped out of the study before attending any sessions due to scheduling conflicts, and one male participant dropped out of the study halfway through due to dislike of mushrooms. Thirty-two participants (17 women, 15 men) completed the entire study. The University of Minnesota Institutional Review Board Human Subjects Committee reviewed and approved all methods for human participants, and all participants provided written informed consent.

Experimental Design and Treatments

This study used a randomized, open-label crossover design to assess the difference on gut health outcomes of protein-matched amounts of mushrooms and meat. The amounts of mushrooms and meat (93% lean ground beef) were matched for protein because this experiment was also part of a satiety study conducted in our laboratory.[318] Each serving of mushrooms also contained approximately 6 g of candidate prebiotics (Table 5-1), exceeding the 3 g per day identified as the minimum oral dose required to elicit an effect by the International Scientific Association for Probiotics and Prebiotics.[36]

Participants completed one in-person visit at the beginning of each experimental treatment (mushrooms and meat). At in-person visits, participants were given breakfast sandwiches containing mushrooms (226 g) or meat (28 g).[318] Gastrointestinal tolerance and breath hydrogen were assessed at baseline and at regular intervals throughout each 3.5 hour in-person visit. Upon leaving the in-person study visit, participants were given a serving of mushrooms or meat to consume at dinner that night as well as at breakfast and

at dinner for the following nine days (Table 4-3). Participants performed a five-day total fecal collection the last five days (days 6 to 10) of each treatment period.

Gastrointestinal Tolerance

Participants completed gastrointestinal (GI) tolerance questionnaires on three days of each ten-day feeding treatment. At each in-person visit, participants completed questionnaires at baseline, and at 60, 120, and 180 minutes after baseline as well as 12 hours after baseline (8:00pm). Participants were asked to complete GI tolerance questionnaires at the same times (8:00am, 9:00am, 10:00am, 11:00am, and 8:00pm) on days two and ten of each treatment period.

These questionnaires required participants to rate the severity of specific gastrointestinal symptoms they experienced. Gastrointestinal (GI) tolerance of the mushroom and meat treatments were measured with seven different symptoms (gas/bloating, nausea, flatulence, diarrhea, constipation, GI cramping, GI rumbling). Participants ranked symptom severity using a 4-point Likert scale (“none,” “mild,” “moderate,” and “severe”). While no GI tolerance scale has been validated in a healthy population to our knowledge, this scale has been used in previous studies conducted in our lab to assess tolerance.[19,20,335]

Colonic Fermentation

Breath samples were collected at baseline and at 60, 90, and 180 minutes after baseline. Subjects were asked to fill a sample collection bag (750 mL) with air. All breath samples were analyzed using the same instrument, a BreathTracker (QuinTron Instrument Company, Milwaukee, WI). For analysis, 20 mL of a breath sample was injected into the BreathTracker. Breath hydrogen and breath methane samples were all evaluated twice for each sample for greater accuracy. The two measurements were averaged before computing final results.

Fecal Collection

Participants were given specimen collectors and anaerobic pouches as well as coolers and ice packs to collect samples. Participants were instructed to bring their samples, on ice in insulated coolers, to the lab within 2 hours of defecation.

Samples were processed within one hour of their arrival in the lab. Samples were weighed and assessed for Bristol scale[336] by visual comparison with Bristol scale pictures and written descriptions. Fecal samples were then divided into aliquots for different experiments. Samples for branched-chain fatty acid (BCFA) and SCFA determination were immediately frozen at -80°C until analysis.

Fecal pH

For fecal pH, a 10g aliquot of fresh fecal sample was diluted 1:10 (w/w) with phosphate buffered solution. The fecal/PBS mixture was homogenized in a stomacher for

2 minutes, and the homogenized sample then used for pH measurement using a calibrated pH probe.

BCFA and SCFA analysis

Fecal samples were analyzed for BCFA and SCFA content using the extraction and derivatization procedures described by Han et al.[337] Briefly, 1 g of fecal sample was combined with 10 mL 50% aqueous acetonitrile, and the mixture homogenized with a vortex. Then samples were centrifuged at 4000g at 10℃ for 10 minutes. The clarified extract was then diluted 1:100 with 50% aqueous acetonitrile and 9 µM of internal standard added. Samples were stored at -80℃ until analysis. Before analysis, 20 µL each of 3-Nitrophenylhydrazine hydrochloride solution and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride solution were added to 40 uL of the extracted sample and the mixture incubated at 40℃ for 30 minutes.

Samples (10 µl) for LC-MS/MS Selective Reaction Monitoring (SRM) Analysis of SCFA and BCFA were subjected to separation using an Shimazu UFLCXR system coupled to an analytical Waters Aquity BEHc18, 1.7um, 2.1x50mm column at 50℃ connected to the Applied Biosystem 5500 iontrap fitted with a turbo V electrospray source run in negative mode with declustering potential and collision energies (Supplemental Table S5-1). The samples were subjected to a linear gradient of A: 15% acetonitrile 0.55 formic acid B: 55% Acetonitrile 0.1% formic acid for 12 minutes at a column flow rate of 400 µl /min. The column was cleared with 95% Acetonitrile for 2 minutes and then equilibrated to buffer A for 3 minutes. Transitions monitored as in

Supplemental Table S5-1 were established using the instrument's compound optimization mode with direct injection for each compound. The data was analyzed using MultiQuant™ (ABI Sciex Framingham, MA) providing the peak area. A standard curve was constructed using from picomole to nanomole in 10 µL. Samples were run in duplicate and concentrations determined from the standard curve. SCFA and BCFA concentration values for each participant were averaged across all samples submitted during each treatment.

Statistical Analysis

Our sample size was selected to give us at least 80% power to detect a significant mean difference of 0.7 SD in markers of gut health between the two diets. Paired t tests were conducted to compare the means between the two diets. Analyses were performed using the Statistical Analysis System (SAS, version 9.3, 2011; SAS Institute, Cary, NC). P-values <0.05 were considered statistically significant.

Compliance

Participants were provided tracking checklists to record which days they ate and did not eat provided study foods. Participants were also asked to complete 24-hour food diaries for days 1 (in-person visit), 2, and 10.

Results

Breath Hydrogen

There were no differences in breath hydrogen or in breath methane between the two treatments as assessed with baseline-corrected AUC measurements (Table 5-2).

Gastrointestinal Tolerance

On Day 1, participants reported significantly more total GI symptoms during the mushroom treatment in comparison to the meat treatment (Table 5-3). Significant individual GI symptoms included gas and flatulence only. On Day 2, participants also reported significantly more total GI symptoms, gas, and flatulence during the mushroom treatment. On the last day of eating each study diet (Day 10), there were no significant differences between the two diets with any of the GI symptoms.

Laxation Markers

Participant stool weights were significantly higher on the mushroom treatment than the meat treatment ($p=0.002$) (Table 5-4). However, there were no significant differences between the two diets in terms stool frequency, pH, or consistency (Table 5-4).

SCFA and BCFA Concentrations

There were also no significant differences between acetate, propionate, butyrate, isobutyrate, or valerate concentrations between the two treatments (Table 5-5). There was a significant difference with isovalerate concentration ($p=0.02$), which was higher in the meat control diet.

Compliance

For the mushroom diet, 20 participants turned in fully completed checklists. Five participants turned in mostly complete checklists with five or fewer missed servings. Seven participants turned in blank checklists or were missing checklists altogether. For the meat diet, 22 participants turned in fully complete checklists, four turned in partially completed checklists, and six participants had missing or blank checklists. All participants brought in at least one fecal sample during each collection period. In addition, approximately one-third (31.25%) of participants had visible pieces of mushroom in stool samples at least once during the mushroom treatment, indicating some level of compliance with treatment protocol.

Discussion

The objective of this study was to assess the effect of mushroom consumption compared to a meat control on markers of gut health. While the mushroom treatment

contained low-digestible carbohydrates and the meat treatment did not, there were few differences in impact on gut health markers between the two treatments.

Both treatments were generally well-tolerated by participants in this study, and no adverse symptoms were reported with either treatment. Total GI tolerance scores as well as gas and flatulence ratings were significantly higher on the first two days of the mushroom treatment. The mushroom treatment provided only an additional 6 g of fiber to our participants' diets. However, since study participants were low fiber consumers, even the 6 g addition may have contributed to increased GI symptoms. An intervention trial with legumes found that adding 4-7 g of fiber daily from beans initially caused an increase in perceived flatulence, but GI tolerance scores returned to normal after a few weeks of daily bean consumption.[338] A similar effect may have occurred in our study, as there were no significant differences in GI tolerance ratings on day 10. Participants may have adjusted to eating a large quantity of mushrooms after ten days, or, as reported in our previous publication on this study,[318] participants may have decreased fiber from other sources over the ten-day period, also decreasing their symptoms. Since participants completed subjective questionnaires at the same time on each day of the intervention, GI symptoms could also have been caused by dietary or lifestyle factors besides the study treatments.

Breath hydrogen and breath methane values did not differ significantly between the two treatments. While we did not expect a difference in breath methane, since elevated breath methane values primarily indicate whether an individual is a "methane producer" with colonic methanogenic colonies,[339] the lack of difference in breath

hydrogen measures may be due to a limitation in our methods. The final breath hydrogen measurement in our study was taken 180 minutes after treatment intake, which was likely not sufficient time for transit of our treatment foods to the colon.[340]

Other measures of colonic fermentation, including fecal pH and SCFA concentrations, also did not differ significantly between the two treatments. Colonic fermentation, which leads to the formation of acids, including SCFA, can acidify the stool.[35] The lack of significant difference in either fecal pH values or SCFA concentrations between the two treatments suggests that there were no differences in colonic fermentation.

The SCFA findings do not support our hypothesis. Unlike meat, mushrooms contain resistant starch, which tends to increase amounts of SCFA when it reaches colonic bacteria, according to previous research.[334] However, the presence of undigested mushrooms in some participants' stool indicates that the mushroom treatment was only partially broken down by digestive processes that occur after chewing. Some, if not all, of the resistant starch or other components provided by the mushroom treatment may not have been available to colonic bacteria at all. The assays used to determine the low-digestible carbohydrate content of the mushroom treatment (described in Table 5-1 footnotes) were conducted with roasted mushrooms that had been ground into a well-blended and homogenous sample, which is not reflective of how food is digested *in vivo*.[341,342] The availability of low-digestible carbohydrates in mushrooms may depend on how thoroughly the mushrooms were chewed by participants. The cell wall of mushrooms is made up of insoluble β-glucans[343] and chitin,[344] and humans do not have

digestive enzymes to break down those components. It may be that the degree to which the mushrooms were chewed by participants before being swallowed affected the extent to which these components were fermented in the gut.

With BCFA, there was a significant difference between the treatments with isovalerate concentration, which was higher during the meat diet ($p=0.02$). While SCFA production appears to be largely beneficial and indicates the production of energy for colonocytes, among other benefits,[35,312,345,346] BCFA production indicates proteolysis occurring in the large intestine and is presumed to be detrimental to health.[345,346] BCFA are formed in the gut when branched chain amino acids (valine, leucine, and isoleucine) are metabolized and fermented.[347,348] Isovalerate specifically is formed by the breakdown of leucine. While the amount of meat provided in our experimental treatment was smaller than a typical serving (2 oz/day) and participant diet records indicated no significant differences in protein intake during the two interventions,[318] beef contains much more leucine (1.267 g/100 g)[6] than mushrooms (0.120 g/ 100 g),[6] which may be responsible for the elevated fecal isovalerate concentrations.

According to both the FDA and the Institute of Medicine (IOM), improved laxation, or the elimination of fecal waste, is considered a beneficial physiological effect of fiber intake.[349,350] Both groups consider stool frequency, ease of defecation, and, in some contexts, fecal weight or fecal bulk as markers of improved laxation.[349,350] In this study, mushroom consumption improved laxation as measured by one of these metrics (fecal weight) but not the others (stool frequency and ease of defecation). Stool consistency (ease of defecation) and frequency did not differ between the two diets. Stool

consistency or form measures (Bristol score) estimate whole gut transit time and ease of defecation.[336] While there are no official 'cut-offs' associated with health or unhealthy states with the Bristol scale, scores 3 and 4 are sometimes referred to as "normal" stool types because they are not associated with urgency, straining, or incomplete evacuation.[351] The average stool type for participants on both diets was between a 3 and a 4 on the Bristol scale (i.e. soft, but formed stools), suggesting that both treatments allowed for "normal" laxation. While stool frequency (e.g. number of stools per day) did not differ between the two treatments, in line with our hypothesis, fecal bulk (stool weight) was significantly higher with the mushroom treatment. Yet FDA draft guidance from 2016 states that "an increase in fecal weight does not necessarily indicate improved bowel function."[349] Increased fecal weight may not necessarily indicate improved laxation, but it does indicate the presence of a fiber source "slowly, incompletely, or essentially not fermented in the large intestine,"[352] which aligns with our other findings. The laxation outcomes of this study may also be subject to limitations. While participants were asked to bring in all stool samples for each five-day period within two hours and record defecation time, we do not know if all stool samples were submitted within that time frame or if all samples were submitted.

Conclusions

The results of this study, especially the increase in fecal bulk, the lack of an increase in SCFA production compared to the meat diet, and the presence of undigested mushroom in the stool, suggest that mushrooms may not be fermentable by human

colonic bacteria and that the “low-digestible carbohydrates” in mushrooms may function as non-digestible carbohydrates *in vivo*. Mushrooms may not influence gut health in humans as they do in other animals. Further research is needed to determine whether chewing or other digestive processes increase low-digestible carbohydrate availability of mushrooms.

Table 5-1. Carbohydrate content of roasted *A. bisporus m*ushrooms[§§§§]

Carbohydrate	**Percentage of roasted *A. bisporus* mushrooms**
Total Dietary Fiber	4.9%
Insoluble Dietary Fiber	3.5%
Soluble Dietary Fiber	1.4%
Beta-glucan	1.76%
Mannitol	2.96%
Resistant starch	<2%

[§§§§] Amounts determined by Medallion Laboratories 1/4/17 using AOAC 2011.25 for fiber determination, AOAC: 2022.02 for resistant starch, and an internal method for sugar alcohols determination using the same brand of *A. bisporus* mushrooms (roasted) utilized in the study.

Table 5-2. Breath hydrogen and breath methane results

	Mushroom (N=32)	**Meat (N=32)**	**p-value**
Breath Hydrogen[a]	-12.37 ± 30.83	-12.68 ± 19.77	0.96
Breath Methane[a]	-0.20 ± 10.78	-2.03 ± 7.76	0.41

[a]Mean baseline-adjusted area under the curve ± SD

Table 5-3. Gastrointestinal tolerance measures day 1

GI Symptom	Mushrooms (N=32)	Meat (N=32)	p-value
GI Tolerance[a]	7.88 ± 7.99	4.09 ± 10.63	0.0491
Nausea[a]	0.06 ± 1.27	-0.19 ± 1.01	0.35
Flatulence[a]	2.95 ± 2.95	0.67 ± 2.63	0.0002
Diarrhea[a]	-0.14 ± 0.99	0.19 ± 1.11	0.22
Constipation[a]	0.36 ± 1.43	0.66 ± 2.11	0.52
Gastrointestinal rumbling[a]	0.94 ± 2.46	0.61 ± 3.60	0.64
Gastrointestinal cramping[a]	0.75 ± 2.01	0.80 ± 2.47	0.92
Gas	2.95 ± 4.05	1.36 ± 3.07	0.0452

[a]Mean baseline-adjusted area under the curve ± SEM

Table 5-4. Gastrointestinal tolerance measures day 2

GI Symptom	Mushrooms (N=32)	Meat (N=32)	p-value
GI Tolerance[a]	7.75 ± 8.47	2.95 ± 5.77	0.0042
Nausea[a]	0.28 ± 2.14	0.16 ± 0.92	0.75
Flatulence[a]	2.38 ± 2.84	0.61 ± 2.54	0.0156
Diarrhea[a]	0.58 ± 1.51	0.36 ± 1.00	0.50
Constipation[a]	0.11 ± 0.89	0.66 ± 1.63	0.11
Gastrointestinal rumbling[a]	1.50 ± 3.27	0.47 ± 2.46	0.16
Gastrointestinal cramping[a]	0.86 ± 2.42	0.42 ± 1.85	0.35
Gas	2.05 ± 3.31	0.28 ± 1.55	0.0051

[a]Mean baseline-adjusted area under the curve ± SEM

Table 5-5. Gastrointestinal tolerance measures day 10

GI Symptom	Mushrooms (N=32)	Meat (N=32)	p-value
GI Tolerance[a]	5.59 ± 8.83	3.86 ± 7.67	0.36
Nausea[a]	0.20 ± 0.91	-0.06 ± 0.96	0.094
Flatulence[a]	1.11 ± 2.46	0.55 ± 2.38	0.38
Diarrhea[a]	0.45 ± 1.52	0.22 ± 1.57	0.56
Constipation[a]	0.75 ± 2.23	0.52 ± 1.73	0.65
Gastrointestinal rumbling[a]	1.09 ± 3.15	1.31 ± 3.88	0.70
Gastrointestinal cramping[a]	0.56 ± 2.43	0.27 ± 1.66	0.55
Gas	1.42 ± 2.56	1.06 ± 2.73	0.59

[a]Mean baseline-adjusted area under the curve ± SEM

Table 5-6. Laxation Markers for fecal samples collected days 6 through 10

Laxation Measure	Mushroom (N=32)	Meat (N=32)	p-value
Fecal wet weight (g/stool)[a]	122.42 ± 58.74	94.62 ± 56.58	0.002
Stool consistency[a,b]	3.12 ± 0.89	3.35 ± 0.79	0.11
Fecal pH[a]	6.86 ± 0.21	6.87 ± 0.16	0.77
Stool frequency [a]	4.25 ± 1.30	4.13 ± 1.26	0.35

[a]Data presented are mean values ± standard deviation

[b]Stool consistency rated on Bristol stool scale, where 1= separate hard lumps and 7= entirely liquid

Table 5-7. Fecal short chain fatty acids (SCFA) and branched chain fatty acids (BCFA) produced on mushroom and meat diets

SCFA and BCFA (uM/mL)	Mushrooms (N=32)	Meat (N=32)	p-value
Acetate[a]	3930.35 ± 732.34	3825.01 ± 731.01	0.16
Propionate[a]	64.72 ± 45.04	64.73 ± 34.07	0.99
Butyrate[a]	53.81 ± 37.69	58.27 ± 33.43	0.46
Isobutyrate[a]	28.42 ± 25.68	30.76 ± 25.49	0.52
Valerate[a]	8.02 ± 5.36	9.78 ± 6.30	0.10
Isovalerate[a]	5.31 ± 2.86	6.92 ± 3.52	0.02

[a]Mean values ± SD on a wet matter basis

Supplemental Table S5-1. Declustering potential and collision

Fatty Acids	Q1 M/Z	Q2 M/Z	Dwell Time	Decluster Potential	Collision Energy	Retention Time
Acetate	194	137.1	50	-40	-20	2.8
Propionate	208	165.1	50	-50	-20	4.9
Isobutyrate	222	137.1	50	-65	-20	8.0
4-Methylvalerate	250	137.1	50	-65	-20	12.2
Valerate	236.104	137.1	50	-65	-20	10.0
Butyrate	222.08	137.1	50	-65	-20	8.3
2-Ethylbutyric acid	250.1	137.1	50	-65	-20	12.5
Isovalerate	236.103	137.1	50	-65	-20	10.0

Section 3: Snacks

Chapter 6: Healthy Snacks: Using Nutrient Profiling to Evaluate the Nutrient-Density of Common Snacks in the United States

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Summary

The objective was to quantify and compare the nutrient-density of commonly consumed snacks using two nutrient-density measures, Nutrient Rich Foods Indices 9.3 (NRF 9.3) and 15.3 (NRF 15.3). Our study design was to identify commonly consumed categories of snacks and individual snack foods, calculate NRF 9.3 and 15.3 scores, rank snacks by category and by individual food based on nutrient density, and compare and contrast scores generated by the two NRF Indices. NRF 9.3 and 15.3 scores were the main outcome measures. The averages and standard deviations of nutrient-density scores for each snack category were used in our analysis. Vegetables and coffee/tea received the highest category scores on both indices. Cakes/cookies/pastries and sweets had the lowest category scores. NRF 9.3 scores for individual snacks ranged from –46 (soda) to 524 (coffee). NRF 15.3 scores ranged from –45 (soda) to 736 (coffee). If added to food labels, NRF scores could help consumers identify more nutritious choices. The differences between NRF 9.3 and 15.3 scores generated for the same foods and the limitations of

these indices highlight the need for careful consideration of which nutrient-density measure to include on food labels as well as consumer education.

Introduction

Most Americans over the age of 2 eat at least 1 snack a day[97] and receive roughly a quarter of their daily energy from snacks.[69] Yet, in part because so little information is available about "snacking" as an eating behavior, the health impacts of snack consumption remain largely unknown. Snacking has been associated with promoting both overweight/obesity and healthy weight maintenance.[353,354] Other research shows no association between snacking and weight.[355] Recent studies suggest that the impact of snacking on adiposity is due primarily to snack selection rather than snack frequency, time of day, or even energy content.[76,356] In addition, snacking has been associated with improved diet quality[83,86,357] and increased micronutrient intake.[357] However, all of these associations between snacks and health outcomes depend upon the definition of "snack" used, and there is a considerable variety of definitions used in research.[84] Snacks have been defined by the "time of day of an eating occasion, type of food consumed, amount of food consumed, location of food consumption, or a combination of several of these factors."[354] In addition, especially in large epidemiological studies, participants often self-designate their "meals" and "snacks," while in other projects, researchers classify eating occasions for participants.[354] In part because there is no definition of a "snack," there are few official recommendations for healthy snack selection.[354] Like the word "snack," "healthy" and "healthful" have a plethora of meanings to different consumers

and even among the research community. However, "healthy" also has a specific meaning defined by the FDA.[358] This definition of "healthy" must be fulfilled for food manufacturers to use the term on product labels. Foods with the "healthy" label may not exceed specific thresholds for fat, saturated fat, cholesterol, and sodium content. Yet, this definition is currently being reviewed by the FDA[359] and will likely be changing. Without referencing the FDA's definition of "healthy," the American Heart Association provides a short list of "healthy snacks" on its website[360] but no guidelines for identifying other healthful options. Similarly, the 2015 Dietary Guidelines for Americans (2015 DGA) encourage "nutrient-dense" snacks but provide few examples or recommendations for identifying them.[44]

While the 2015 DGA are intended for a professional audience, not for consumers, their recommendation to choose "nutrient-dense" snacks as a healthful option may be difficult to communicate to consumers without supplying significant nutrition education as well. Previous studies show that consumers have difficulty identifying nutrient-dense foods even after reading food labels.[361] The small number of consumers who read food labels do not tend to read past the first few lines of nutrition facts.[362] Including a measure of nutrient density, especially on the front of food packages,[363,364] may facilitate consumer understanding and clinician encouragement of nutrient-dense choices as well as incentivize food companies to develop more nutrient-dense options.

Several nutrient profiling methods have been proposed in the literature,[365–369] but this study will assess the nutrient density of common snack foods using two different versions of the Nutrient Rich Foods (NRF) Index. Like some other nutrient density

measures,[366,369] NRF index scores are positively correlated with the 2005 Healthy Eating Index, a diet quality scale developed by the USDA.[370] In contrast with other nutrient profiling methods, however, the NRF index scoring procedure is non-propriety. In addition, also unlike other scoring systems including the Nutritional Quality Index, the Nutritious Food Index, the NuVal system, Guiding Stars, and Smart Choices, the NRF scoring system does not include dietary cholesterol as a nutrient to limit in its calculations.[366,367,369,371,372] According to the 2015 DGA, there is no longer a recommended limit of 300 mg of dietary cholesterol daily.[44] While these new dietary guidelines do recommend avoiding foods high in both cholesterol and saturated fatty acids, without a recommended limit, there is no longer a need for a daily value. Therefore, while developed several years ago, the NRF index reflects the most recent views of the nutrition science community.

Five different versions of the NRF index have been published: NRF 6.3, 9.3, 10.3, 11.3, and 15.3.[370,373] These versions primarily differ in the number of "nutrients to encourage" that each one considers. The number of "nutrients to encourage" is designated by the first number in the index names (i.e. 6, 9, 10, 11, 15). For example, NRF 9.3 includes the following nine nutrients to encourage: protein, fiber, calcium, iron, magnesium, potassium and vitamins A, C, and E. This list of nutrients reflects the nutrients of concern identified by the 2005 DGA, the most recent DGA released when the NRF was developed in 2008.[374,375] While these nutrient profiling measures were developed nearly a decade ago, all of the nutrients of concern identified in the 2005 DGA are still listed as underconsumed in the 2015 DGA.[44] NRF 15.3 has a similar list of

nutrients to encourage but includes a few additional nutrients to encourage (monounsaturated fat, vitamin D, thiamin, riboflavin, B-12, and folate) and excludes magnesium.[370] The three "nutrients to limit" are the same for all of the NRF indices and include saturated fat, added sugar, and sodium.[370] USDA dietary guidelines have recommended against frequent consumption of these three nutrients since the 1980s.[44,93,374,376–380]

NRF 9.3 and 15.3 were used for this study, because the NRF 9.3 scores have the highest correlation with HEI scores,[370] and NRF 15.3 includes all of the nutrients of public health concern identified in the most recent DGA (calcium, potassium, fiber, and vitamin D).[44] NRF scores can be calculated per 100 kcal of a food, per 100g of a food, or per serving size (reference amount customarily consumed, or RACC) of a food.[370,381] For consistency across all food and beverage items, this study calculates NRF values per 100 kcal.[382]

NRF Index scores are calculated by summing the "percent daily values" for nutrients to encourage and subtracting from this sum the "percent daily values" for nutrients to limit per 100 kcal of a food or beverage. "Percent daily values" are generated by dividing the amount of a certain nutrient in 100 kcal of a food or beverage by the amount of that nutrient recommended for daily consumption (i.e. the Daily Reference Value, or DRV) as part of a 2,000 calorie diet by the Institute of Medicine.[383] The quotient is then multiplied by 100 to generate a percent daily value. For example, 100 calories of crackers contain 0.75 mg of iron. The DRV for iron is 18 mg, therefore, crackers contain 4.17% of the daily recommendation of iron. Once a percent daily value

has been calculated for each nutrient needed for NRF index calculations, the percent daily values for nutrients to encourage are added together and the percent daily values of nutrients to limit are added together. The sum of nutrients to limit is then subtracted from the sum of nutrients to encourage, resulting a single number, the NRF index score. High scores indicate nutrient-density, and low scores indicate poor nutrient-density. Tables 6-1 and 6-2 show sample calculations for NRF Indices 9.3 and 15.3, respectively.

Our objective was to calculate and compare the nutrient density scores of popular snacks using NRF 9.3 and 15.3. While the 2015 DGA,[44] among other sources,[384,385] identify snacking as a rapidly increasing habit among Americans, snacking patterns and choices are heterogeneous and vary by age, development stage, gender,[355] and even geographic location.[354] This study uses commonly consumed snacking categories identified by Nicklas and others[357] using National Health and Nutrition Examination Survey (NHANES) data from 2001-2008. While the NHANES data used in this study is also from a heterogeneous group, it is limited to data from adult participants ages 19 and older (n= 18,988).[357] The group was 50% female and excluded pregnant and lactating females.[357]

The word "snack" in this paper is defined the same way it is in NHANES surveys, namely as an "eating occasions with foods or beverages not consumed with meals."[357] This definition was used in NHANES surveys for subjects to self-designate eating occasions.

Based on previous publications, we hypothesize that some of the snack categories and individual snack foods that American adults consume will receive high scores on

both indices, despite the common perception that snack foods are rich in nutrients to limit.[64,70,72,76,77,87,354,384]

In addition, comparing the scores generated by NRF 9.3 and 15.3 will showcase the malleability of nutrient profiling scoring systems and the importance of carefully selecting which nutrients are included in nutrient density calculations. For instance, foods made with refined flours will likely have higher scores on the NRF 15.3 index due to fortification rather than inherent nutrient density.

Materials and Methods

To calculate and compare the nutrient densities of different snacks, first we identified common categories of snack foods consumed in the U.S. Next, we identified specific snacks to analyze within each category. Then we calculated NRF 9.3 and 15.3 scores for each specific snack. Finally, we calculated an average nutrient density score and standard deviations for each category of snacks with both NRF 9.3 and 15.3.

Identification of Common Snack Foods

In their analysis of data from the 2001-2008 National Health and Nutrition Examination Survey (NHANES), Nicklas, O'Neil, and others[357] describe eleven "clusters," or categories, of common snack choices for the U.S. population. These categories and their relative popularity as indicated by the percentage of individuals listing these foods as snacks are cakes/cookies/pastries (12%), sweets (9%), vegetables

(8%), alcohol (8%), milk desserts (8%), crackers/salty snacks (7%), soft drinks (6%), other grains (6%), whole fruit (4%), coffee/tea (2%), and "miscellaneous" (17%).[357] The miscellaneous category of snack foods includes low fat milk, cheese, meat/poultry/fish, cakes/cookies/pastries, crackers/salty snacks, fruit juice, and fruit drinks.[357] In their analysis, Nicklas, O'Neil, and others[357] also identify an additional "no snacks" cluster encompassing 13% of the NHANES population. The NHANES data used to generate these snacking categories originated from the 24h diet records of adults ages 19 and older who were non-pregnant and non-lactating.

NRF index scores can only be generated for individual foods, and we selected representative foods for nutrient density calculations within each of these snack categories. Because Nicklas, O'Neil, and others[357] identified seven specific foods within the "miscellaneous" category, these foods were selected for this category. For the categories of cakes/cookies/pastries, sweets, vegetables, alcohol, milk desserts, crackers/salty snacks, other grains, whole fruit, three specific products were used for analysis in each category. For the coffee/tea category, four products were used to reflect sweetened and unsweetened coffee and sweetened and unsweetened tea. For the soft drink category, only two products were used to reflect regular soft drinks and diet soft drinks. The specific products used for each category are described on Table 6-3 and were selected due to their popularity among U.S. consumers as reported in scientific literature, government databases, and published market research information. Table 6-3 also lists the sources used to identify each product or group of products.

Nutrient Data

After identifying specific snacks, nutrient data for each food was obtained from the Nutrition Data System for Research (NDSR) software version 2014 developed by the Nutrition Coordinating Center at the University of Minnesota, Minneapolis, MN. As shown in Table 6-3, the nutrient data for most products analyzed in this study were from "generic," rather than branded, products. In NDSR, when a product brand is "unknown," NDSR defaults to the nutrient information for whichever selection is most common. For instance, if butter popcorn is selected but the salt level is unknown, "NDSR will by default provide the nutrient data for butter popcorn with salt, since salted buttered popcorn is more commonly selected than unsalted buttered popcorn" (personal correspondence, NDSR Support).

NRF Index 9.3 and 15.3 Calculations

Microsoft Excel (Version 2010, Microsoft, Inc.) was used to calculate NRF 9.3 and 15.3 scores for each product. For a few foods, the percent daily value of some nutrients exceeded 100%. Fruit juice and tomatoes provided more than 100% of the vitamin C and the riboflavin content in 100 kcal of coffee also exceeded 100% of the daily value. These values were "capped" at 100% so that no percent daily value greater than 100% was included in the NRF scores.

We made slight modifications to the NRF scoring procedures for "added sugars"

and for monounsaturated fats (MUFAs). Both NRF 9.3 and 15.3 utilize "added sugars" values in their calculations. When the NRF Index scoring procedures were originally published in 2008,[370] there was no daily value for added sugars. However, the FDA has recently approved a daily value for added sugars on nutrition facts labels as well as required disclosure of added sugar content. For our NRF Index calculations, therefore, we utilized this daily value for added sugars. Because food companies have until July 2018 to include added sugar values on their nutrition facts labels,[265] the added sugar values used for this analysis were generated by NDSR's reverse-engineering process. "Added" sugar values cannot be determined chemically or analytically, so NDSR's process calculates the added sugar (by total sugars) content of a food using ingredient lists.

In addition, NRF 15.3 scores include monounsaturated fatty acids (MUFA) as nutrients to encourage. There is no established daily value for MUFAs. Previous publications using the NRF 15.3 Index used 20 g as a reference daily value for MUFAs but do not provide a currently accessible reference for this number.[370,381] Therefore, for calculating NRF 15.3 scores in our study, we used the amount of MUFAs required for a health claim on olive oil by the FDA. According to this health claim, eating 23 g of olive oil may "reduce the risk of coronary heart disease due to the MUFAs in olive oil" [386]. In NDSR, 23 g of olive oil contain approximately 16 g of MUFAs. Therefore, we used 16 g as a proxy DRV for MUFAs in our NRF 15.3 calculations.

Results

The overall average NRF 9.3 score was 49 ± 103 and the average NRF 15.3 score

was 86 ± 145. However, when NRF scores greater than 200 (i.e. for black coffee, black tea, and lettuce) were omitted, the average NRF 9.3 score was 26 ± 49 and the average NRF 15.3 score was 51 ± 69. Overall, the snack foods used in this analysis had a very wide range of nutrient densities. Some snacks had NRF scores over 100, while others like ice cream and regular soda actually received "negative" NRF scores, indicating a high amount of nutrients to limit and a low amount of nutrients to encourage.

On both indices, vegetables/legumes and coffee/tea were the most nutrient-dense categories, while cakes/cookies/pastries and sweets were the most nutrient-poor (Table 6-4).

Generally, the relative ranking of snack categories by nutrient density was similar with both NRF 9.3 and NRF 15.3 scores. There were a few slight differences in ranking by category score with the two indices. Milk desserts had a lower nutrient-density score than alcohol on the NRF 9.3 scale but not on the NRF 15.3 scale. Similarly, soft drinks had a lower NRF 9.3 value than salty snacks, but soft drinks had a higher NRF 15.3 value than salty snacks. Finally, "other grains" had a higher NRF 15.3 score than whole fruit, but whole fruit had a higher NRF 9.3 score than "other grains." Because the values of the nutrients to limit for each food were the same with both indices, any differences in scores are due only to different amounts of nutrients to encourage considered for NRF 9.3 versus 15.3. When the nutrient-density "rankings" of the snack categories by NRF 9.3 and by NRF 15.3 scales are considered side by side, none of the categories moves by more than one position relative to its position on the other list.

NRF 9.3 and 15.3 had huge ranges for individual snacks. NRF 9.3 values ranged

from -46 (regular soda) to 524 (black coffee). Similarly, NRF 15.3 values ranged from -46 (regular soda) to 736 (black coffee). The full list of nutrient-density scores for individual snacks can be found on Table 6-5. Cola, sweetened tea, and cake were the most nutrient-poor foods on both the NRF 9.3 and NRF 15.3 indices. Black coffee, lettuce, and black tea had the highest NRF scores on both indices. When scores greater than 200 were omitted, the ranking of individual snacks varied by index. For NRF 9.3, fruit juice, onions, shrimp, chicken, and cheese had the highest scores. For NRF 15.3, shrimp, fruit juice, low-fat milk, onions, and chicken had the highest scores.

Some of the differences between NRF 9.3 and NRF 15.3 rankings of individual snacks is due to products containing enriched flour, which has some nutrients like riboflavin and thiamin that contribute to NRF 15.3 scores only. The inclusion of these vitamins increased the NRF 15.3 scores of several food items, including pretzels, cookies, cakes, and ready-to-eat cereal. In addition, some dairy foods like low-fat milk and pudding received higher scores with the NRF 15.3 index because vitamin D, B_{12}, zinc, and riboflavin, all naturally present in or added to dairy foods, were included on that scale only.

When assessed by relative popularity and nutrient-density, the most commonly selected snack category, “miscellaneous,” also received some of the highest nutrient-density scores. However, the cakes/cookies/pastry category, the third most commonly selected, also received the third lowest scores on NRF 9.3, which does not consider several nutrients in enriched flour.

Discussion

American adults already choose to consume some nutrient-dense snacks, like vegetables, low-fat milk, cheese, meat/poultry/fish, and fruit. Other commonly consumed snacks, notably soft drinks, candy, and cake, have low nutrient-density. Reputable sources of nutrition information, including the 2015 DGA, convey similar information and recommend consuming more vegetables, low-fat dairy, and lean meats and avoiding added sugars and excess amounts of refined grains.[44] Nutrient-dense scoring may provide redundant information about these foods. However, some snacks fall in between the extremes, and there is very little guidance for consumers in this realm. In this analysis, some of the foods between the nutrient-dense and nutrient-poor ends of the "nutrient-density spectrum" included fruit drinks, potatoes, chips, wine, popcorn, and beer. Consumers could use nutrient-dense scoring with items like these to identify more nutrient-dense choices.

NRF Index scores provide valuable information not easily available from other sources, including current nutrition labels. For the first time in twenty years, the FDA introduced changes to the Nutrition Facts label in 2015.[265] Notable changes include labeling added sugar content and requiring a larger type size for "calories," "servings per container," and "serving size."[358] While these changes emphasize the caloric and sugar content of foods, they do not change the ability of consumers to determine the nutrient density of their food choices, a focus of the 2015 DGA.[44] For example, both pretzels and pistachios have a serving size (reference amount customarily consumed) of 30 g.[14] While pistachios are more nutrient dense than pretzels (NRF 9.3 scores of 26 and 4,

respectively), the new labels will show in large bold font that the pretzels contain about 60 fewer calories per serving than pistachios [6]. Although the new labels also require that the amounts of all nutrients of public health concern (calcium, vitamin D, fiber, and potassium) identified in the 2015 DGA be listed, the predominant focus on calories seems contradictory to DGA recommendations in this case. While the DGA encourage nutrient density, nutrient adequacy, and healthy eating patterns, the new labels from the FDA seem to suggest that energy content, the largest and most easily visible piece of information on the new labels, is more important.[265]

In addition, though most U.S. grocery shoppers report wanting to improve their health by purchasing "healthier" foods,[387] few consumers read nutrition facts panels at all. Recently published research suggests that color-coded front-of-pack (FOP) nutrition labels may draw more consumer attention than nutrition facts panels and, therefore, represent a better way to highlight nutrition information.[363,364] While there are currently no standards for FOP labels, the FDA began an initiative to develop FOP standards in October 2015.[388] Including a measure of nutrient density on the new FOP labels could simplify snack selection by allowing consumers to compare foods using a metric besides calories or nutrients to limit, the focus of many current FOP labels.[364] Research and consumer testing are needed with the standardization process for FOP labels to ensure that new labeling measures will promote consumer use[363] and consumer understanding of overall nutrient composition.[389] While nutrient profiling is already provided for some foods voluntarily by grocery stores,[390] the usefulness of these scoring systems to consumers still remains relatively unexplored.[391,392]

However, the current constraints of nutrition labels suggest that nutrient profiling information that allows consumers to easily compare foods by their overall nutrient composition would be valuable. Consumers have many facts to consider when selecting foods. Marketing and consumer research argues that consumers must balance three different types of "costs" to incorporate nutrition into their food purchasing considerations.[387] When shopping, consumers must balance the costs of acquiring nutrition information, comprehending and interpreting nutrition information, and synthesizing all of the information about a product (including price and nutrients) to make a decision.[387] While nutrition facts labels have eliminated the cost of acquiring nutrition information, consumers still have to interpret that information and incorporate it into purchasing decisions. Nutrient content claims (e.g. reduced fat, high fiber) provide consumers with basic interpretations of nutrition facts panels, but like nutrition facts panels, nutrient content claims highlight certain attributes of a food and do not reflect its overall nutrient profile. Implementing a consistent form of nutrient profiling on FOP labels may reduce the comprehension and evaluation costs to consumers and may, therefore, help some consumers make better selections.

Yet, nutrient profiling, including for use on product labels, has considerable limitations.[372] In our analysis, for instance, some of the snacks with the highest scores on NRF 9.3 and 15.3, like black coffee and tea, are not actually nutrient dense. Obtaining the nutrients in 100 kcal of black coffee would require drinking 50 cups, which is physiologically improbable. Similarly, some other snacks like diet soda rank "higher" in nutrient-density than foods like whole fruit and low-fat dairy, which contradicts most

dietary advice from reputable sources, including the 2015 DGA. Diet soda has an NRF 9.3 value of 64, which is higher than that of a banana (53) or low-fat milk (60). According to NDSR software, diet soda contains 12 kcal per serving. To obtain the nutrients in 100 kcal of diet soda would require drinking 8.3 servings. In contrast, a single banana is 100 kcal and has a similar NRF score. A few other foods, including vegetables like lettuce, are similar to diet soda in this respect. While these foods have very high NRF scores, it is unlikely that 100 kcal of these foods would be consumed in one sitting. The artificially elevated scores of energy-poor foods are important limitations to the use of nutrient-density scoring per 100 kcal of a food. While it was outside the scope of this paper to conduct a parallel analysis of nutrient density using serving sizes or 100 g amounts, these methods also have limitations and impact nutrient profiling.[375,381]

In addition, the NRF scores generated by this study also do not necessarily reflect the typical serving method for these foods. Lettuce may be a commonly consumed vegetable because it is eaten in sandwiches, salads, or with other foods, not because it is eaten alone. However, due to limited information on how lettuce is consumed as a snack, plain, undressed lettuce was used for NRF 9.3 and 15.3 scoring in this study. The preparation method is unknown for many of the foods on this list and is an important limitation to our study results. Finally, nutrient density measures cannot reflect bioavailability of different nutrients, nor can they account for variations among foods that may be induced by storage, growth conditions, and preparation methods.

Using nutrient profiling to assess nutrient density is also challenging because the term “nutrient-dense” has not been formally defined or validated by the nutrition research

community. While the DGA have proffered some definition of the term "nutrient-dense" since 2005, these definitions rely on comparisons between different foods and remain imprecise.[44,93,374] The 2005 DGA[374] first defined nutrient-dense foods as "foods that provide substantial amounts of vitamins and minerals and relatively fewer calories." The 2010 DGA further expanded on this term by adding that nutrient-dense foods contained calories not "diluted" by added solid fats, added sugars, added refined starches, or by naturally occurring solid fats.[93] The 2010 DGA also labeled specific, entire categories of food as "nutrient-dense" including vegetables, fruits, whole grains, seafood, eggs, beans and peas, unsalted nuts and seeds, fat-free and low-fat milk and milk products, and lean meats and poultry.[93] The 2015 DGA echoes the 2010 DGA definition. These 2010 and 2015 DGA definitions do not account for differences within food categories and only allow for categories of food to be "ranked" as more or less nutrient-dense rather than individual foods.[393] These current definitions also prohibit the creation of quantitative cut-offs values for "nutrient-dense" and "nutrient-poor" foods.

Like nutrition facts panels, nutrient profiling reflects the contents of the food not the context in which they are consumed or prepared. Yet, even though nutrient profiling can only be applied to individual foods rather than an overall dietary pattern, nutrient profiling may help consumers to interpret nutrition information about foods and, depending on the profiling method, allows consumers to compare foods within and among different categories. Nutrient profiling in its current iteration does have caveats that prevent it from being immediately implementable on nutrient labels. However, the nutrition research community has already identified many of the current research gaps

preventing implementation, including identifying a unified definition for “snacks” and of “nutrient density” and conducting consumer research to assess the potential impact and utility of including nutrient profile information on FOP labels.

Conclusions

The snacks with the highest and lowest nutrient-density scores in this analysis are, respectively, already being recommended or discouraged by reputable nutrition authorities. Nutrient-density scores may not provide new information about snacks at either end of a “nutrient-density spectrum,” but if added to food labels, they could serve as helpful tools for consumers trying to identify more nutritious options among the foods located between the extremes. The differences between NRF 9.3 and 15.3 scores generated for the same food and the limitations of these indices highlight the need for careful consideration of which nutrient-profiling measure to include on food labels as well as consumer education.

Table 6-1. Sample calculation of a NRF 9.3 Index score

Nutrients	**Amount in 100 kcal**	**Daily Reference Value**	**Percent Daily Value**	
Calcium (mg)	18.93	1000	1.89	Sum of nutrients to encourage: 16.18
Fiber (g)	0.42	25	1.68	
Iron (mg)	0.75	18	4.17	
Magnesium (mg)	3.292	40	0.82	
Potassium (mg)	21.81	3500	0.62	
Protein (g)	1.44	50	2.88	
Vitamin A (IU)	0	5000	0	
Vitamin C (mg)	0	60	0	
Vitamin E (IU)	1.23	30	4.12	
Added Sugars (g)	1.43	50	2.86	Sum of nutrients to limit: 13.92
Saturated Fat (g)	0.98	20	4.90	
Sodium (mg)	147.74	2400	6.16	
NRF 9.3 Score:				2.26

Table 6-2. Sample calculation of a NRF 15.3 Index score

Nutrients	**Amount in 100 kcal**	**Daily Reference Value**	**Percent Daily Value**	
Calcium (mg)	18.93	1000	1.89	Sum of nutrients to encourage: 41.12
Fiber (g)	0.42	25	1.68	
Folate (µg)	28.40	400	7.10	
Iron (mg)	0.75	18	4.17	
Monounsaturated fat (g)	1.05	16	6.56	
Potassium (mg)	21.81	3500	0.62	
Protein (g)	1.44	50	2.88	
Riboflavin (mg)	0.07	1.7	4.12	
Thiamin (mg)	0.11	1.5	7.33	
Vitamin A (IU)	0	5000	0	
Vitamin B-12 (µg)	0	6	0	
Vitamin C (mg)	0	60	0	
Vitamin D (IU)	0	400	0	
Vitamin E (IU)	1.23	30	4.10	
Zinc (mg)	0.10	15	0.67	
Added Sugars (g)	1.43	50	2.86	Sum of nutrients to

Saturated Fat (g)	0.98	20	4.90	limit: 13.92
Sodium (mg)	147.74	2400	6.16	
NRF 15.3 Score:				27.2

Table 6-3. Description of each food item used for NRF Index scoring by category of commonly consumed snacks

Snack Category	Food	Food Description in the Nutrition Data System for Research Database
Miscellaneous Snacks	Low fat milk [394]	Milk, 1% fat or low-fat
	Cheese[394]	Cheese, unknown variety of natural cheese, unknown type
	Beef[394]	Beef, unknown kind of beef
	Chicken[394]	Chicken, unknown if light or dark meat, unknown if skin eaten
	Shrimp[395,396]	Shrimp, unknown if cooked from fresh, frozen, or canned
	Fruit drink[394]	Juice or flavored drink, unknown fruit or flavor, drink
	Fruit juice[394]	Juice or flavored drink, orange, juice, unknown type
Cakes/Cookies/Pastries	Pastry[394]	Pies, snack- commercial, fruit-filled, apple
	Cookies[394]	Cookies and bars, unknown type
	Cake[394]	Cake, unknown type, frosting-unknown kind
Sweets	Milk chocolate	Chocolate candy, chocolate candy bar,

	bar [397]	milk, plain, regular
	Peanut butter cups[397]	Candy, Reese's® Peanut Butter Cup
	Candy coated chocolates[397]	Chocolate candy, chocolate coated or covered pieces, candy coated, plain, unknown if milk or dark chocolate
Vegetables/Legumes	Potatoes[398]	Potato, unknown if baked, boiled, canned, or roasted, unknown if with skin, salt-regular, unknown if fat used
	Lettuce[398]	Lettuce, unknown type
	Onion[398]	White, yellow or red, cooked, unknown preparation
Alcohol	Beer[399]	Beer, unknown type
	Wine[399]	Wine, other table (<15% alcohol), red
	Hard liquor[399]	Liquor, unknown type
Milk Desserts*****	Ice cream	Ice cream and frozen desserts, unknown % fat, vanilla or other flavors (include chocolate chip)
	Pudding	Other flavors, unknown type, prepared
	Frozen yogurt	Ice cream and frozen desserts, frozen

***** No lists available

		yogurt, vanilla or other flavors, unknown % fat
Crackers/Salty Snacks	Crackers[400]	Crackers, unknown type
	Pretzels[400]	Pretzels, unknown type, unknown if salted, sticks
	Chips[400]	Chips-snack type, potato, unknown type, unknown if salted, unknown if regular or thick cut, ingredient fat not known
Soft Drinks†††††	Cola	Cola, regular, unknown if with caffeine
	Diet cola	Cola, diet, unknown if with caffeine, unknown artificial sweetener
Other Grains‡‡‡‡‡	Popcorn	Popcorn, unknown type, unknown if topped with fat or salt
	Granola bar	Granola bars, unknown type
	Ready-to-eat cereal	Cereal, ready-to-eat, unknown type
Whole Fruit	Banana[398,401]	Banana, fresh or ripe
	Apple[398,401]	Apple, fresh, with skin

††††† No lists available

‡‡‡‡‡ No lists available

	Grapes[398,401]	Grapes, fresh
Coffee/Tea[§§§§§]	Coffee	Coffee, unknown type, unknown preparation
	Coffee	Coffee, regular (caffeinated), made from ground, cream (unknown if regular or fat free), sugar, white granulated
	Tea	Tea, brewed (from tea leaves or tea bag)- all flavors or plain, regular, unsweetened
	Ready to drink sweetened tea	Tea, purchased ready-to-drink- all flavors or plain, unknown if regular or decaffeinated, unknown if sweetened

[§§§§§] No lists available

Table 6-4. NRF 9.3 and 15.3 average scores for each category of snack food and overall average

Snack Category	**NRF 9.3 Score**	**NRF 15.3 Score**
Alcohol	7	9
Cakes/cookies/pastries	-16	2
Coffee/Tea	175	281
Crackers/Salty Snacks	10	33
Milk desserts	-11	13
Miscellaneous	82	118
Other Grains	33	101
Soft Drinks	9	47
Sweets	-13	4
Vegetables	129	162
Whole Fruit	44	51
Overall Average ± St. Dev.	49 ± 103	86 ± 145

Table 6-5. NRF 9.3 and NRF 15.3 Index scores for individual foods

Snack Food	NRF 9.3 Value	NRF 15.3 Value
Apple	49	53
Banana	53	59
Beef	10	67
Beer	8	13
Black coffee	524	736
Black tea	212	388
Cake	-31	-17
Candy coated chocolates	-11	1
Cheese	101	131
Chicken	116	142
Chips	23	35
Cola	-46	-46
Cookies	-10	8
Crackers	2	27
Diet cola	64	139
Frozen yogurt	-7	10
Fruit drink	39	41
Fruit juice	128	144
Granola bar	-2	7
Grapes	30	41

Hard liquor	0	0
Ice cream	-8	14
Lettuce	226	302
Low fat milk	60	144
Milk chocolate bar	-18	-3
Onion	126	142
Pastry	-6	16
Peanut butter cups	-9	12
Popcorn	8	18
Potatoes	35	43
Pretzels	4	36
Pudding	-17	15
RTD sweetened coffee	10	39
RTD sweetened tea	-45	-39
RTE cereal	94	278
Shrimp	120	156
Wine	12	12

Chapter 7: The Nutrient Density of Snacks: A Comparison of Nutrient Profiles of Popular Snack Foods Using the Nutrient-Rich Foods Index

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Summary

Although Americans receive almost a quarter of their daily energy from snacks, snacking remains a poorly defined and understood eating occasion. However, there is little dietary guidance about choosing snacks. Families, clinicians, and researchers need a comprehensive approach to assessing their nutritional value. The objective is to quantify and compare the nutrient density of commonly consumed snacks by their overall nutrient profiles using the Nutrient-Rich Foods (NRF) Index 10.3. Our method was to calculate NRF Index scores for the top 3 selling products (based on 2014 market research data) in different snack categories. These NRF scores were averaged to provide an overall nutrient-density score for each category. Based on NRF scores, yogurt (55.3), milk (52.5), and fruit (30.1) emerged as the most nutrient-dense snacks. Ice cream (−4.4), pies and cakes (−11.1), and carbonated soft drinks (−17.2) emerged as the most nutrient-poor snacks. The NRF Index is a useful tool for assessing the overall nutritional value of snacks based on nutrients to limit and nutrients to encourage.

Introduction

Although current cross-sectional data suggest that most Americans, including children and adolescents, consume a significant portion of their daily energy as snacks, snacking remains a poorly understood behavior.[402] There is little information on how and why individuals and families select snacks, and no consistent definition of "snacks" or "snacking" used by most consumers or even the research community.[84,354] Many studies, including the National Health and Nutrition Examination Surveys, rely on participants to define "snacks" themselves.[384] While some individuals define snacks as an eating occasion between meals, others define snacks based on the type of food consumed, location of food consumption, or time of day of consumption.[354] Unlike other eating occasion labels like breakfast, lunch, or dinner, "snacks" commonly describes a type of food as well as an eating occasion. Even dietary guidance is prone to inconsistency in defining snacking. The 2015 Dietary Guidelines for Americans, for instance, caution against excessive consumption of "snacks," with regard to the type of food, because they add sugars and saturated fat to the American diet, but they recommend snacks as an eating occasion, suggesting carrots with hummus as a sample "snack meal."[44]

Based on consumer definitions, however, Americans receive a quarter of their daily energy from snacks.[384] The 2015 Scientific Report of the Dietary Guidelines Committee states that 96% of the US population over the age of 2 years eats at least one snack every day[97] and that daily consumption of 2 to 3 snacks is even more common. The results of 2 recent studies suggest that the type of snack, rather than the frequency of consuming snacks, is the most important determinant of whether snack consumption is

associated with adiposity, diet quality, or body mass index.[356,403] However, the term "snacking" is still often associated with the consumption of foods high in saturated fat, sugar, and sodium,[87] commonly referred to as "snack foods."[356,384,403]

While "snack foods" are often associated with nutrients to limit, like most foods, "snacks" also include nutrients to encourage. Yet guidance about overall nutrient composition and the nutrient density of snacks remains largely unavailable. Food labels, for example, draw consumer attention to the calorie and fat content (perceived by many to be less healthful nutrients) at the top of the label but not to the same food's other nutrients such as calcium, potassium, and fiber, listed further down the label. Consumers who read labels, including adults who purchase snacks for their children, tend to read only the first 5 components (servings, calories, total fat, saturated fat, and *trans* fat) of the nutrition facts label, none of which are nutrients to encourage.[362] This may explain why label reading does not necessarily lead to the selection of foods high in nutrients to encourage.[361] Comprehensive dietary guidance about common snack choices based on nutrient density would be useful for different stakeholders. With this guidance, parents could more easily identify healthful snacks for their children, clinicians would have reliable information for counseling patients about snacking and dietary needs, and researchers would be able to assess more easily the impact of dietary trends or interventions that involve snacking.

The purpose of our study was to quantify the nutrient density of commonly consumed snacks using the Nutrient-Rich Foods (NRF) Index, and therefore fill an important need by showcasing a way to assess the nutritional value of snacks, which

make up a large part of the diets of children and families.[203] For the purposes of this article, snacks are defined as food or caloric beverages consumed between regular meals (breakfast, lunch, and dinner). The NRF Index assigns scores to foods based on their nutrients to encourage (protein, calcium, vitamin D, potassium, magnesium, iron, vitamin A, vitamin C, vitamin E, and fiber) and nutrients to limit (sodium, saturated fat, and total sugar). Higher scores indicate more nutrient-dense foods.

Materials and Methods

We obtained data on the most commonly consumed snack categories in the United States from the 2014 National Eating Trends (NET) survey administered in paper form by the NPD Group, a market research company. The NET survey includes data from roughly 5000 individuals annually, 23% of whom are children. NET participants are recruited from a national mail panel, and the main food preparer/purchaser in each household (panelist) records the food and beverage consumption of all household members for a 2-week period. Panelists could record up to 3 snacks (defined as a between meal eating occasion) and up to 3 meals per individual per day.[404] Although families enrolled in the NET are nationally representative in many ways, including geographic distribution, survey participants are, in general, better educated than Americans as a whole. For example, roughly 46% of main food preparers/purchasers have a college degree compared with about 33% of Americans as whole.[405] Hispanics and African Americans are also underrepresented in the sample compared to the US population, making up just 7.9% and 5.8% of participants, respectively, compared with 13.3% and

17.6% of Americans as a whole.[406] Next, we obtained brand information for the 3 market leaders in each snack category identified from the NET based on 2014-2015 sales data from Information Resources, Inc (IRI; http://www.iriworldwide.com/en-US). Table 7-1 includes a list of leading brands and specific products selected for analysis. Table 7-1 does not list nonbranded products (fruit and some varieties of milk). The nonbranded types of milk most commonly consumed for snacks were 2% milk and whole milk, and the most popular types of fruit selected for snacks were apples, bananas, and grapes. Table 7-1 also does not include "private label" top sellers. If 1 of the 3 market leaders was identified as "private label" in the IRI data, a generic version of the product (i.e., "chocolate chip cookies") was selected from the nutrient database (described below) in lieu of a branded product. Nutrient data for snacks were obtained from the Nutrition Data System for Research software, version 2014, developed by the Nutrition Coordinating Center at the University of Minnesota, Minneapolis, MN. This software includes nutrition information on several branded food products. When nutrient details for specific branded foods were not available in this database, we obtained nutrient information by contacting manufacturers directly. We calculated NRF scores for each product and for each snack food category using Microsoft Excel (Version 2010, Microsoft, Inc, Redmond, WA). A few food items included in this analysis, namely, diet cola, sugar free gum, and brewed tea (from tea bags), contain no calories or very few calories in each serving and were excluded from our calculations. Finally, we calculated nutrient-density scores for each food. There are several versions of the NRF Index.[370,407] This study uses a modified version of the NRF Index 9.3, to which we have added vitamin D (listed as a nutrient to

encourage in the 2010 and 2015 Dietary Guidelines for Americans).[44,93] We have designated this vitamin D–augmented version of the NRF Index as "NRF 10.3." First, for each 100 kcal of a specific food, the amount of each nutrient to encourage was expressed as a percentage of its daily recommended value.[383] These percentage values were added together. Next, for 100 kcal of the same food, the amount of each nutrient to limit was calculated as a percentage of the recommended limit. These percentage values were also added together. The NRF Index was then calculated as the sum of the values for nutrients to encourage minus the sum of the values of nutrients to limit. Table 7-2 provides an example. For the NRF Index calculations in this study, we chose to incorporate total sugar values as opposed to added sugar values. It is often difficult or impossible based on common data sources to accurately distinguish between added and total sugars for many snack foods.[408]

Results

Fruit, selected as a snack by 48% of NET respondents in the 2-week survey period, was the most popular snack and had an NRF Index score of 30.1. Cookies, chips, and ice cream followed in popularity, selected by 44%, 33%, and 33%, respectively (Table 7-3). Among the most popular snack categories, NRF scores varied from −17 to 55 (Table 7-4). Yogurt, milk, and fruit were the most nutrient-dense snack categories, while ice cream, pies and cakes, and carbonated regular soft drinks were the most nutrient-poor snacks. The median NRF score for all snack options assessed was 6.0. With

the breadth of scores (−17 to 76) for individual snacks, the mean NRF score for commonly consumed snacks was 12.6 ± 24.1.

Potato chips had a surprisingly high score (19.3). While chips are commonly considered a food high in nutrients to limit, potatoes naturally contain potassium, magnesium, fiber, and vitamin C, and the oil used in chip production adds vitamin E. In addition, chip companies have transitioned to vegetable oils in recent years, limiting saturated fat content.

The snacks in the categories with the highest nutrient density, namely, yogurt and milk, contain high amounts of nutrients to encourage, especially protein, calcium, potassium, vitamin D, and magnesium, with relatively small amounts of nutrients to limit (saturated fat, total sugars, and sodium) in a 100 kcal serving. Yogurt scored higher than milk in this analysis because the leading yogurt products are all non-fat, which has less saturated fat than the market-leading milk varieties (2% and whole). Both yogurt and milk do have relatively low amounts of iron, vitamin A, vitamin C, vitamin E, and fiber. Fruit, the third most nutrient-dense category, contains high amounts of vitamin C, fiber, potassium, and magnesium, and relatively low amounts of protein, calcium, vitamin D, vitamin A, vitamin E, and iron. Compared with yogurt and milk, fruit has a higher total sugar content (a “nutrient to limit” in this analysis), which decreased its NRF score.

The most nutrient-dense snacks, milk and yogurt, were also the least frequently consumed. Only 21% of consumers recorded milk for a snack, and a mere 14% of respondents ate yogurt.

Discussion

Snacks are often considered "unhealthy" foods. Based on NRF scoring, however, this generalization is inaccurate. Several of the foods evaluated in this analysis, including all of the yogurt products, milks, fruits, nuts and seeds, and potato chips had relatively high NRF Index scores, indicating nutrient density. Other frequently selected snacks including soft drinks, pies and cakes, ice cream, and cookies had negative NRF scores and, therefore, low nutrient density.

A narrow focus on one component of a food obscures its overall nutritional value. Flavored milk, for example, contains more added and total sugars than plain milk, but is also rich in calcium and vitamin D, both of which are nutrients to encourage. Unfortunately, current dietary recommendations adopt this narrow view. The 2015 Dietary Guidelines for Americans recommend "choosing nutrient-dense foods and beverages" and then define these foods and beverages as containing "little or no solid fats and added sugars, refined starches, and sodium" but mention no specific nutrients to encourage.[44] Evaluating the nutritional value of any food based only on its contribution of nutrients to limit is unreasonable.[409]

Our analysis provides a more balanced analysis of the nutritional value of commonly consumed snacks but is prone to several limitations. The NRF Index has inherent limitations. Weighing nutrients equally as in the NRF Index calculations may not be a valid method for assessing overall nutritional value. It is not clear to what degree each nutrient to encourage or nutrient to limit contributes to or detracts from health or the overall nutritional value of a food. Weighing nutrients equally also cannot account for

interactions among different nutrients. For example, dietary fat promotes absorption of vitamin D.[410]

Conclusions

The NRF Index is a useful, though imperfect, tool for a more balanced understanding of commonly consumed snacks in the United States. Physicians, dietitians, and other clinicians faced with the challenging task of providing brief counseling on diet and exercise to children and their parents could use the NRF Index to discuss specific snack foods based on their overall nutrient profiles.

Table 7-1. Market-leading snack selections

Snack Category	**Market Leader (Brand)**	**Product**
Candy	Hershey's	Hershey's Milk Chocolate Bar
	M&M	M&M's Peanut
	Trident Sugarless Gum	Trident Spearmint Gum
Pies and cakes	Betty Crocker	Supermoist Yellow Regular Cake Mix
	Duncan Hines	Classic Yellow Regular Cake Mix
	Entenmanns	All Butter Pound Cake
Carbonated soft drinks	Coca-Cola	Coca-Cola
	Diet Coke	Diet Coke
	Pepsi	Pepsi
Chips	Lay's	Lay's Potato Chips (classic)
	Pringles	Pringles Potato Crisps (original)
	Ruffles	Ruffles Potato Chips (original)
Cookies	Nabisco Chips Ahoy	Nabisco Chips Ahoy (original chocolate chip)
	Nabisco Oreo	Nabisco Oreo Chocolate Sandwich (original)
Crackers	Sunshine Cheez-It	Cheez-It (original)
	Pepperidge Farm Goldfish	Goldfish (original)
	Nabisco Ritz	Ritz (original)

Ice Cream	Breyers	Breyers Frozen Natural Vanilla Ice Cream (regular)
	Ben & Jerry's	Frozen Half-Baked Ice Cream (regular)
Milk	Dairy Pure	2% Milk
Popcorn	Orville Redenbacher's Microwave Popcorn	Orville Redenbacher's Pop Up Bowl Microwave Popcorn
	Pop Secret Microwave Popcorn	Pop Secret Movie Theater Butter Microwave Popcorn
Snack nuts and seeds	Planters	Regular Deluxe Mixed Nuts (sea salt, whole and halves, plastic jar)
	Wonderful	Wonderful regular pistachios, salted
Tea	Lipton	Diet green tea with citrus liquid prepared tea with caffeine
	Arizona	Green tea with ginseng and honey (prepared plastic tea jug)
	Lipton	Tea natural black tea bags
Yogurt	Chobani	Chobani Regular Nonfat Plain
	Dannon	Dannon Light N Fit Vanilla Yogurt

Table 7-2. Nutrient-Rich Foods Index 10.3 sample score Calculation for apples

Nutrients	**Amount in 100 kcal of Apples**	**Daily Reference Value**	**Percent Daily Value**	
Protein (g)	0.50	50	1.00	Sum of nutrients to encourage: 48.56
Calcium (mg)	11.32	1000	1.13	
Vitamin D (IU)	0	400	0	
Potassium (mg)	206.13	3500	5.89	
Magnesium (mg)	9.43	40	2.36	
Iron (mg)	0.23	18	1.28	
Vitamin A (IU)	103.77	5000	2.08	
Vitamin C (mg)	8.86	60	14.76	
Vitamin E (IU)	0.47	30	1.57	
Fiber (g)	4.62	25	18.49	
Saturated fat (g)	0.05	20	0.25	Sum of nutrients to limit: 16.34
Sodium (mg)	1.89	2400	0.08	
Total Sugars (g)[a]	20.01	125	16.01	
Nutrient-Rich Foods Index score:				32.22

[a]No daily value for total sugars. The Daily Reference Value used here (125 g) was adopted from an overview of the Nutrient-Rich Foods Index.

Table 7-3. Popularity of snack categories

Category	Percentage of Individuals Selecting at Least Once in 2-Week Period
Fruit	48%
Cookies	44%
Chips	33%
Ice cream	33%
Candy/gum	32%
Popcorn	29%
Carbonated soft drinks	28%
Crackers	25%
Cake	24%
Milk	21%
Nuts/seeds	16%
Tea	15%
Yogurt	14%

Table 7-4. NRF 10.3 scores for snack categories

Category	NRF 10.3 Score
Yogurt	55.3
Milk	52.5
Fruit	30.1
Nuts and seeds	26.7
Chips	19.3
Tea	12.3
Crackers	5.5
Popcorn	1.4
Cookies	–2.1
Candy/gum	–4.0
Ice cream	–4.4
Pies and cakes	–11.1
Carbonated drinks	–17.2

Abbreviation: NRF, Nutrient-Rich Foods Index.

Chapter 8: Snacking for a Cause: Nutritional Insufficiencies and Excesses of U.S. Children, a Critical Review of Food Consumption Patterns and Macronutrient and Micronutrient Intake of U.S. Children

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Summary

The objective of this review was to identify dietary insufficiencies and excesses in children aged two to 11 in the U.S. and eating habits that merit concern in terms of nutrient and energy density to improve overall diet quality. Data from the What We Eat in America (WWEIA) tables from the National Health and Nutrition Examination Survey (NHANES) were examined as well as survey data from the School Nutrition Dietary Assessment Study (SNDA). Analysis of survey data revealed that children consume insufficient Vitamin D, calcium, and potassium and excess energy, carbohydrates, and sodium. Dietary modifications are necessary to prevent serious deficiencies and the development of chronic illness. Snacking has steadily increased in this population since the 1970s, and snacks provide necessary nutrients. However, carbohydrates and added sugars tend to be over-consumed at snacking occasions. Replacement of current snack choices with nutrient-dense foods could lower the risks of nutrient deficiencies and help

lower excess nutrient consumption. Increased consumption of low sugar dairy foods, especially yogurt, at snack times could increase intake of important micronutrients without contributing to dietary excesses.

Introduction

The U.S. has the third highest healthcare expenditures in the world.[411] In 2010, over 17% of the U.S. gross domestic product was spent on healthcare.[412] Preventative care measures, including dietary improvements, could help reduce these costs over time, especially through encouraging health-promoting practices for children. Over 30% of U.S. children and adolescents are overweight or obese,[413] which increases their risk of becoming overweight or obese adults.[414] However, even though they consume excess energy, American children consume insufficient nutrients.

The National Health and Nutrition Examination Survey (NHANES) evaluates the health and diet of the U.S. population. NHANES data show that children aged two to 11 have low overall intakes of fiber, Vitamin D, calcium, and potassium, but an excess consumption of added sugars and refined carbohydrates, in addition to energy. Too little Vitamin D, calcium, and potassium can lead to a wide range of health problems later in life, including osteoporosis, hyperparathyroidism[219] and hypertension.[415] Energy and refined carbohydrate-laden diets can also lead to an increased susceptibility to becoming overweight or obese and to developing cardiovascular disease.[416,417] Furthermore, nutrient-poor diets consumed during childhood can establish a lifetime pattern of poor eating habits.[93]

To improve the health of the U.S. population, it is vital to address the nutrition of its children and promote positive change in children's eating choices. The 2010 Dietary Guidelines for Americans (DGA) provide the official nutrition recommendations for Americans two years of age and older and include information on how to structure a health-promoting diet. The 2010 DGA identify potassium, dietary fiber, calcium, and Vitamin D as "nutrients of concern" across the population.[93] The intake levels of these nutrients are low enough across the population to be a concern for public health;[93] data from the 2009–2010 NHANES also show low intake levels of these four nutrients among children aged two to 11[418] (the only exception to the low levels of nutrients was calcium consumption for two to five year olds, which slightly exceeded the recommended—Dietary Reference Intake). In conjunction with the "nutrients of concern," the 2010 DGA include a list of foods Americans should consume more frequently.[93] This list includes a variety of fruits and vegetables, whole grains, low fat milk and milk products, seafood and other lean protein options, and oils.[93] Vegetables, fruits, dairy products, and whole grains are all mentioned as good food sources for the nutrients of concern.[93] In addition to nutrients of concern and foods to eat more of, the 2010 DGA list food components to reduce, which include sodium, saturated fat, trans fat, cholesterol, added sugar, solid fat, and refined grains.[93]

MyPlate, the visual representation of the 2010 DGA, is another source for U.S. dietary recommendations. The MyPlate graphic is a plate divided into sections by food group (vegetables, fruits, grains, protein foods, and dairy).[3] The size of each section on the plate depicts how much of each food group should be consumed daily. For example,

vegetables and grains are the largest two sections on the plate, reflecting recommendations that Americans consume more vegetables and more whole grains.[3] MyPlate also encourages Americans to be physically active and to avoid dietary sources of solid fats, added sugars, and excess sodium.[3]

In addition to providing information regarding the overall daily intakes of each food group, NHANES data also include information on when children tend to consume different nutrients. Encouraging changes to the nutrient profile of different eating occasions is one possible method of improving children's eating habits.

Materials and Methods

Data Sources

The dietary data used for this analysis come primarily from the What We Eat in America (WWEIA) tables of the 2009–2010 NHANES and School Nutrition Dietary Assessment Study (SNDA) data. NHANES is an annual survey of American children and adults conducted by the Centers for Disease Control and Prevention, the United States Department of Agriculture (USDA), and the National Center for Health Statistics to study the health of the national population, including its dietary habits. WWEIA data are cross-sectional data based on two days of 24-h dietary recall. The NHANES data used originates from the WWEIA tables available on the USDA website and from published scientific articles. Publications used include Centers for Disease Control and Prevention and USDA publications as well as articles found via keyword searches on government

institution websites and scientific databases such as PubMed and ScienceDirect. NHANES 2009–2010 data was the source of information for children's breakfast, lunch, snack, and dinner consumption habits.

Information on children's lunches also comes from the SNDA, which evaluates the nutritional quality of meals offered to school-aged children and adolescents through the National School Lunch Program (NSLP) and the impact of this program on children's health. The USDA's Food and Nutrition Service contracted with Mathematica Policy Research to conduct the SNDA-IV.[419] The SNDA has been administered periodically since 1991.[420] Data from SNDA-III (2004–2005) and SNDA-IV (2009–2010) were used in this publication. SNDA data were used in conjunction with NHANES data for lunch consumption information because many elementary school students (63%) participate in the NSLP.[420] The NSLP follows recommendations outlined in the 1995 School Meals Initiative for Healthy Children (SMI). SMI recommendations are based on Recommended Daily Allowances and the DGA.[420] SMI identifies "target nutrients" for school lunches, including protein, vitamin A, vitamin C, calcium, and iron.[420] The SNDA distinguishes between foods "offered" versus foods "served" at school lunches. Foods offered accounts for all options available for students to choose. Foods served includes the foods that students select or are given for lunch.

Food consumption and macro- and micronutrient intake of children aged two to 11 from WWEIA tables and the SNDA-IV were compared with existing dietary recommendations to determine inconsistencies between recommendation and practice. Macronutrients studied include energy, carbohydrates, protein, sugar, fiber, and fat.

Micronutrients studied include calcium, vitamin D, vitamin B12, magnesium, sodium, and potassium. NHANES data, SNDA-IV data, data from scientific publications, and recommendation data was reformatted into tables and graphs using Microsoft Excel.

Dietary recommendations were taken from the USDA publications of the 2010 DGA and choosemyplate.gov website as well as from publications from the Institute of Medicine (IOM) for vitamin D and calcium intake recommendations. The 2010 DGA are largely based on 2005–2006 NHANES data and the IOM's Dietary Reference Intakes.

WWEIA information is listed in tables on the USDA website showing the relative percentage and amount of each nutrient consumed. These data are organized by gender, age group, and ethnicity. Energy contributions of each eating occasion (breakfast, lunch, snacks, and dinner) as well as nutrient consumption at each eating occasion are also available on the website.

For children aged two to five (n=861),[418] a parent or caregiver completed the dietary recall information. For children aged six to 11 (n=1132),[418] a parent or caregiver assisted the child with completion of the dietary intake questions.

School lunch data from the SNDA-IV is available online in summary reports. NSLP data from 902 schools, including 316 elementary schools, was collected for the SNDA-IV.[419] Schools across the country were selected for the survey to produce nationally representative data. Data was collected both from School Food Authorities (SFA), or school district groups, and from groupings of SFAs and schools.[419] All public SFAs that participated in the NSLP were considered for participation in the SNDA-IV.[419] SFAs and schools were selected via two sample frames.[419] A sample of SFAs was chosen

first.[419] Then, from a second sample of additional SFAs, individual schools were selected for the study based on location, income level, urbanicity, number of students, and SFA size.[419] Two surveys were administered, one to the SFA-only sample and a second survey for the SFA plus schools sample. A total of 595 SFAs were recruited for the study. Five hundred and seventy-eight SFAs completed SFA-level Director Survey.[419]

Results and Discussion

Children's Food Intake by Meal

Our results represent information from the primary data sets listed above. Food intake data in children is limited, and the data sets used for our analysis are considered the best information available on eating habits and nutrient intakes of children in the U.S. Self-report data have limitations, but for these surveys, attempts were made to obtain the best information possible. For example, pre-school children are not able to describe their food intake accurately, so parents or caregivers completed the dietary recalls for children aged two to five years.

Furthermore, different surveys use different cut-off points for data analysis. Since our information was obtained from published papers and government reports, we were unable to correct for different age ranges. We always point out the age range used in the data set we are quoting.

NHANES surveyed children aged two to 11 years and grouped them into "younger" (ages 2–5) and "older" (ages 6–11) categories. Some reports such as DGA list

individuals up to 13 years of age as children and split the children into three groups by age range. Different studies may also include different exclusion criteria, but the goal of these surveys is to obtain a representative sample of American children.

Breakfast: Most children consume at least one food for breakfast, and foods eaten at breakfast provide important micronutrients, especially Vitamin D and calcium.[421] NHANES data shows that breakfast provides 34%–39% of children's Vitamin D intake and 25%to 28% of their calcium intake.[421] Overall, breakfast contributes to 30%–35% of total daily key vitamin and mineral intake (with "key vitamins and minerals" defined as Vitamin D, B12, calcium, sodium, potassium, and magnesium).[421]

Snacks: Ninety-seven percent of the children surveyed eat a snack, and half of these children eat multiple snacks per day.[402] Snacks contribute to 37% of children's energy intake[385] but only provide 15%–30% of vital micronutrients.[422] Popular snack choices include desserts and sugar-sweetened drinks,[385] and snacks consist of almost 40% of the added sugar in children's diets.[422] Overall, children aged two to five consume 12 teaspoons of sugar per day, and children aged six to 11 consume about 18 teaspoons of sugar per day.[418]

Lunch: SNDA data shows that the average school lunch comes within 10% of the SMI's standards for its target nutrients.[423] NSLP lunches also generally provided at least one third of the recommended daily amounts of grains, dairy foods, and oil, but they are also high in calories from solid fats and added sugars.[420]

According to NHANES data, most children (93%) aged two to five eat lunch, and lunch provides about 25% of their daily energy intake.[424] Lunch accounts for 23% of the

consumption of nutrients of concern for these children and also contributes to 20% of their sugar intake.[424]

Dinner: Most children consume at least one food item for dinner, an eating occasion that provides 21%–28% of calcium intake and about 20% of Vitamin D, 30% of potassium, and 30% of the dietary fiber consumed by children aged two to 11.[425] Dinner foods also comprise about 20% of children's sugar intake.[425]

Children's Overall Nutrient Intake

Most American children consume snacks, but most of the snacks consumed are energy-rich and nutrient-poor choices, especially considering that children already consume excess energy and insufficient nutrients. Although snacking itself can be an important habit for weight maintenance,[426] replacing current snack foods with health-promoting options, especially options naturally abundant in nutrients of concern, would improve children's diet quality.[427]

Certain nutrients of concern, notably calcium and Vitamin D, are sometimes consumed as supplements, however, the Food and Drug Administration, the Academy of Nutrition and Dietetics, and the 2010 DGA recommend consuming foods for adequate nutrition instead of supplements.[93] Based on this recommendation, improving children's nutrient intake would be best accomplished through changing food consumption habits rather than encouraging supplement usage or reliance. Children already receive most of their calcium and potassium intake from food instead of supplements; more than 97% of

calcium consumed by children comes from food alone[428] and almost 100% of children's potassium intake[428] comes from food.

Yogurt, fruits, and vegetables are naturally rich sources of the 2010 DGA's nutrients of concern and are also foods that children do not consume sufficiently (Table 8-1).[93] Adding one 6 oz serving of yogurt each day would help children move closer to DGA recommendations for almost all of the nutrients of concern;[429] combining yogurt and fruit or yogurt and vegetables for snacks would increase consumption of all of the calcium, 301 mg potassium, 2.2 μg Vitamin D, and 31 g sugar.[6] Adding one daily serving of yogurt as a snack for children ages nine to 11 would provide enough calcium for this group to meet recommended intake levels (Table 8-2), and a serving of yogurt would increase Vitamin D and potassium consumption for children in all age groups. Children of all age groups do not consume enough of those two nutrients (Table 8-2).

Yogurt manufacturers are already working to decrease the amount of added sugars in yogurt. The amount of added sugars listed by the USDA's National Nutrient Database for Standard Reference,[6] referenced above, does not reflect the amount of added sugars in some yogurt brands. For 6 oz of low fat strawberry yogurt, one national brand contained 21 g of sugar and another national brand contained 24 g, while some yogurt marketed to children can have as few as 18 g of sugar, according to company websites. The amount of sugar in these products is much lower than the amount listed in the USDA's database. However, not all yogurts on the market have reduced sugar content, and many products still do not meet the IOM's standard for competitive foods in schools. Although removing all added sugars would likely discourage consumption, especially

among children, establishing the IOM's 23 g of total sugar per 6 oz serving as a recommended maximum amount would allow for a decrease in added sugars without removing them completely.

Finally, the proposed Nutrition Facts labels would decrease the serving size, or Reference Amounts Customarily Consumed, of yogurt from 8 oz to 6 oz,[430] a more common size for single-serving yogurt containers.[430] This serving size reduction would allow low fat yogurt to contain 3 g of fat per 6 oz of yogurt instead of 3 g of fat per 8 oz serving. Recent studies suggest that dairy fat and full-fat dairy products confer health benefits that low-fat or fat-free dairy products do not.[207,270,431] Choosing a whole milk yogurt instead of a fat-free yogurt may also decrease the need for added sugars to increase palatability, as the fat in yogurt enhances flavor perception and increases satiety. In comparison to a 6 oz serving of fruit-flavored yogurt, 6 oz of plain whole milk yogurt contains a total of 8 g of sugar with amounts of vitamin D (if fortified), calcium, and potassium comparable to that of a low fat, flavored yogurt.[6] Increasing acceptability and availability of whole milk yogurts with low sugar content may be a beneficial way to encourage health-promoting and nutrient-dense snacking among children without raising sugar consumption.

Limitations of the Review

Using different sources for intake and recommendation information created some limitations to the scope and analysis of this review. As shown in Tables 8-1 and 8-2, data sources for nutritional intake and recommendation information use different age ranges,

which complicates comparisons. NHANES, for example, separates data about children into “two to five years old” and “six to 11 years old” categories, while the 2010 DGA has three categories for children: one to three years old, four to eight years old, and nine to 13 years old.

In addition, because data for lunch consumption in this paper comes from NHANES data for children ages five years of age and younger and from school lunch intake data (SNDA-IV) for children ages six and older, and different data are collected for each survey, developing a comprehensive overview of children’s lunch consumption habits was not feasible. For example, the SNDA does not collect data on all of the DGA’s nutrients of concern, including Vitamin D and potassium. This difference may be a cause for concern because it prohibits the development of a complete overview of the nutrient consumption of children while at school and complicates finding and addressing the most prevalent areas of concern in children’s diets.

School lunch data presents an additional complicating factor, because SNDA data reflect the meals that children were offered or served but not consumption data. While children may be offered or served certain foods, data on food consumption is not collected. A recent study on school lunch waste indicated that the measure of food served was not an adequate representation of food consumption at school lunch.[432]

Finally, when the data analysis for this review was conducted, only raw data from the 2009–2010 NHANES were available. Analyzed data from the 2009-2010 NHANES were not yet available.

Conclusions

American children aged two to 11 consume extra energy and sugars in their diets but insufficient Vitamin D, calcium, and potassium. One way to address the insufficiencies and excesses of children's diets would be to change the nutrient density of children's snacks. Foods high in added sugars and energy currently dominate children's snack choices. Substituting one serving of low sugar, whole milk yogurt, paired with fruit or vegetables, for current snacks would increase children's consumption of valuable nutrients without adding excess sugar or energy.

Table 8-1. NHANES food groups: recommended intake versus actual consumption, comparisons using recommendation information from choosemyplate.gov and consumption data from 2009–2010 NHANES data for children 2–11 years old

Food Group[433]	Recommended Daily Intake[3]	Actual Intake[433]
Dairy foods	2–3 years old (both genders): 2 c 4–8 years old (both genders): 2.5 c 9–13 years old (both genders): 3 c	2–5 years (females): 2.46 c 2–5 years (males): 2.31 c 6–11 years (females): 2.03 c 6–11 years (males): 2.46 c
Fruits	2–3 years old (both genders): 1 c 4–8 years old (both genders): 1- 1.5 c 9–13 years old (females): 1.5 c 9–13 years old (males): 1.5 c	2–5 years (females): 1.43 c 2–5 years (males): 1.49 c 6–11 years (females): 1.20 c 6–11 years (males): 1.03 c
Protein foods	2–3 years old (both genders): 2 oz 4–8 years old (both genders): 4 oz 9–13 years old (females): 5 oz 9–13 years old (males): 5 oz	2–5 years (females): 2.93 oz 2–5 years (males): 3.05 oz 6–11 years (females): 3.59 oz 6–11 years (males): 3.97 oz
Vegetables:	2–3 years old (both genders): 1 c 4–8 years old (both genders): 1.5 c 9–13 years old (females): 2 c 9–13 years old (males): 2.5 c	2–5 years (females): 0.69 c 2–5 years (males): 0.66 c 6–11 years (females): 0.80 c 6–11 years (males): 0.78 c

Total Grains: refined and whole grains	2–3 years old (both genders): 3 oz 4–8 years old (both genders): 5 oz 9–13 years old (females): 5 oz 9–13 years old (males): 6 oz	2–5 years (females): 4.54 oz 2–5 years (males): 4.92 oz 6–11 years (females): 6.73 oz 6–11 years (males): 6.75 oz
Whole Grains	2–3 years old (both genders): 1.5 oz 3–8 years old (both genders): 2.5 oz 9–13 years old (females): 3 oz 9–13 years old (males): 3 oz	2–5 years (females): 0.61 oz 2–5 years (males): 0.79 oz 6–11 years (females): 0.61 oz 6–11 years (males): 0.65 oz

Table 8-2. 2010 DGA nutrients of concern: recommended intake versus actual consumption, comparisons using Dietary Reference Intakes or Adequate Intake and 2009–2010 NHANES data for children 2-11 years old

Nutrient of Concern	Recommended Daily Consumption	Actual Daily Intake (from Food)
Vitamin D	1–3 years old (both genders)[434]: 10 μg 4–8 years old (both genders)[434]: 10 μg 9–13 years old (both genders)[434]: 10 μg	2–5 years old (both genders)[428]: 6.8 μg 6–11 years old (both genders)[428]: 6.1 μg
Potassium	1–3 years old (both genders)[434]: 3000 mg 4–8 years old (both genders)[434]: 3800 mg 9–13 years old (both genders)[434]: 4500 mg	2–5 years old (both genders)[428]: 2071 mg 6–11 years old (both genders)[428]: 2172 mg
Calcium	1–3 years old (both genders)[434]: 700 mg 4–8 years old (both genders)[434]: 1000 mg 9–13 years old (both genders)[434]:	2–5 years old[428]: 1032 mg 6–11 years old (both genders)[428]: 1048 mg

	1300 mg	
Dietary fiber	1–3 years old (both genders)[434]: 19 g 4–8 years old (both genders)[434]: 25 g 9–13 years old (females)[434]: 26 g 9–13 years old (males)[434]: 31 g	2–5 years old (females)[418]: 11.3 g 2–5 years old (males)[418]: 12.1 g 6–11 years old (females)[418]: 14.5 g 6–11 years old (males)[418]: 13.6 g

Section 4: Dairy Foods

Chapter 9: Defining "Protein" Foods- Why Not Dairy?

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Summary

Changing the name of the "protein foods" group on the U.S. Department of Agriculture's (USDA) visual food guide, MyPlate, back to the "meat & beans" group would provide important clarification regarding USDA recommendations for a balanced diet. Previous iterations of the food guide named the protein group after its constituent foods (i.e. the "meat & beans" group on the 2005 MyPyramid), and the reasons for renaming the entire group with MyPlate are unclear.

The exclusion of dairy foods from the "protein foods" group of 2010 MyPlate illustrates the shortcomings of this group's name. Dairy foods contain high-quality, affordable protein and constitute a significant portion of the protein intake among the U.S. population but are not listed as "protein foods" on MyPlate. Dairy products and other high-calcium foods do have their own section of MyPlate; however, having this separate group does not mitigate the disingenuousness of having a "protein group" that excludes an important protein source. Additionally, since consumers tend to understand food-based terms better than nutrient-based terms, a change to "meat & beans" group

would also provide clarification for consumers and for educators regarding the content and role of this group.

Introduction

Changing the name of the "protein foods" group on the U.S. Department of Agriculture's (USDA) visual food guide, MyPlate, back to the "meat & beans" group would provide important clarification regarding USDA recommendations for a balanced diet. The name "protein foods" is confusing because this group excludes protein-rich dairy foods and its name refers to a nutrient instead of foods.

Dairy foods, an important source of high-quality, affordable protein for Americans,[203,435,436] are not included in this group.[3] Although dairy has its own section of MyPlate, this section focuses primarily on the calcium content of dairy foods, not their protein content.[437] Because dairy foods constitute a substantial portion of protein consumed by the U.S. population,[435,436] they belong in a group titled "protein foods." Yet the nutrient profile of dairy foods is markedly different from the profiles of the current "protein foods" like meat, legumes, poultry, and eggs. While adding dairy foods to the current "protein foods" group could displace important nutrients like iron and B vitamins, leaving dairy foods out of the protein group downplays their considerable protein quality. Renaming the "protein foods" group to reflect its constituent foods would clarify this inconsistency.

In addition, renaming the "protein foods" the "meat & beans" group would aid consumer understanding of MyPlate. Historically, groups on the USDA food guides have

been named after foods instead of nutrients,[438] and consumers understand this food-based terminology better than nutrient names.[439] MyPlate is intended for consumer use and education and needs to contain accurate information that is accessible and understandable to consumers.

Dairy Foods: Protein Content, Quality, and Role in the American Diet

By excluding dairy, the "protein foods" group name of MyPlate neglects the contribution of dairy foods to protein intake in the U.S. For many Americans, dairy is an important dietary staple and major source of protein.[435,436] Milk is the primary source of protein for children ages two to 18, comprising 13.2% of the total protein this group consumes.[435] Among Americans ages 19 to 50, cheese and milk are the third and fourth most common food sources of protein, respectively.[436] Milk also provides 7.4% of protein for adults over the age of 51[436] and is among the top five sources of dietary protein for Americans along with poultry, meats, mixed dishes (meat, poultry, fish), and bread.[440] Yet while poultry, meats, and fish are included in the "protein foods" group, bread and milk are not. Bread and milk do contain less protein per gram than poultry, meats, and fish. Grilled chicken breast meat has about 31 g of protein in every 100 g, and cooked ground beef (90% lean) has 26 g of protein in every 100 g of beef.[6] Yogurt and milk, on the other hand, both contain slightly over 3 g of protein in every 100 g, and wheat bread has roughly 11 g of protein per 100 g.[6] However, protein amount does not reflect protein quality. Protein sources contain different combinations of amino acids and

are not equally digestible. In terms of quality, milk is among the best protein sources. It contains all nine essential amino acids in a bioavailable and digestible form.[25,202]

According to the most common protein quality evaluation measure, the Protein Digestibility Corrected Amino Acid Score (PDCAAS), milk protein has a value of 1.00, the highest possible quality rating on this scale.[24] Historically, protein quality evaluation measures have even used milk protein as a reference to measure the quality of other proteins against.[24,202] PDCAAS scoring evaluates protein quality based on limiting amino acids, fecal digestibility, and the protein needs of preschool-aged children.[24] Yet, because this scale truncates scores greater than 1.00, it still cannot fully reflect the quality of the protein that milk contains. Milk's "true" PDCAAS score is 1.21, but because its concentration of some indispensable amino acids is higher than the amount of those amino acids required by preschool aged children, its value is truncated to 1.00. However, the value of 1.00 versus 1.21 is only relevant in the context of a milk-only diet. As soon as other protein sources are added, which may be deficient in amino acids of which milk has an overabundance, milk can complement those deficient protein sources. Most diets contain a variety of foods, so milk's non-truncated PDCAAS score may more accurately reflect the quality of dairy protein. Table 9-1 lists the PDCAAS values for common protein group foods as well as for wheat and milk. Milk has a PDCAAS value higher than either ground beef or soy, and its non-truncated value is even higher than that of eggs.[25] According to the PDCAAS scale, dairy is a source of excellent protein.

Cost of Dairy Protein and Importance for Vulnerable Populations

In addition, dairy protein is more affordable than some other "protein foods." Generally, protein tends to be more expensive than other sources of calories.[203] Animal protein, which is of higher quality than plant protein, is one of the most expensive foods besides produce.[203] A 2010 study assessed foods from the USDA Food and Nutrition Database and the USDA Center for Nutrition Policy and Promotion's food price database for the nutrient value of foods relative to their cost using the Nutrient Rich Foods Index.[203] This index was used to rate the nutrient density of individual foods by assessing the presence of nutrients to encourage (defined as protein, fiber, Vitamin A, Vitamin C, Vitamin E, calcium, iron, magnesium, and potassium) versus nutrients to limit (added sugars, sodium, and saturated fat).[203] In this study, eggs and milk were among the lowest cost sources of Vitamin A, dietary calcium, Vitamin B_{12}, and riboflavin ($P<0.01$). Eggs and milk were also among the least expensive protein sources.[203] However, while eggs are considered "protein foods" on MyPlate, dairy foods are not.

The lower cost of dairy protein could have important ramifications for vulnerable populations, such as children and older adults, who have high protein needs. While the Recommended Dietary Allowance (RDA) of protein for adults is 0.8 g/kg body weight, the RDA for children ages 1-14 ranges from 1.00 g/kg to 1.14 g/kg.[180] Though not reflected in the RDAs, elderly members of the population may also need more than 0.8 g/kg of protein to slow the loss of muscle tissue and function due to aging.[201] While protein intake is sufficient for most of the population, the 2015 Dietary Guidelines Advisory Committee noted in their report that "6 percent of men older than 80 years and

11 percent of women older than 80 years had protein intakes that were below the protein [Estimated Average Requirement]."[97] Since older adults also have lower energy needs and tend to consume less protein,[201] it is especially important for this group to select foods with high quality protein.

Why not add dairy to the protein group?

Because of their high-quality protein content and contribution to the protein intake of Americans, dairy foods are "protein foods."[44,97] However, dairy does not belong with the other "protein foods," because of its very different nutrient profile. In addition, dairy has its own section of MyPlate.[441]

In addition to protein, dairy contains calcium, Vitamin D, and potassium, three of the nutrients of concern identified in the 2015 Dietary Guidelines for Americans (2015 DGA).[44] However, dairy does not contain iron, niacin, Vitamin E, or Vitamin B_6 like meat, poultry, beans, eggs, fish, and nuts and seeds do.[442] Therefore, although dairy foods are considered a food group to increase in the 2015 DGA,[44] adding dairy to the "protein foods" group could result in nutrient displacement if dairy overtakes other sources of protein in the diet.

Furthermore, dairy already has its own unique section on MyPlate. In this section, daily consumption of two to three cups of dairy products is recommended primarily to encourage adequate calcium intake. The only dairy foods included in this group are those dairy products that "retain their calcium" after processing, like milk, cheese, and

yogurt.[437] The dairy group also encompasses non-dairy sources of calcium, including calcium-fortified foods (juices, soymilk, cereals), canned fish, tofu, and leafy greens.[443]

Nutrients versus foods: historical precedence and consumer education

Dairy foods are "protein foods" but differ considerably from the foods currently in the "protein foods" group. MyPlate is the first U.S. food guide to name a major food group with a nutrient.[438] The historical precedent has been to label all food groups by their constituent foods. As shown in Table 9-2, the previous names of the "protein foods" group used terminology similar to "meat & beans." Despite the major name change with MyPlate, the list of foods in this group has hardly changed since 1916 (Table 9-2). A recent commentary addressing MyPlate myths suggests that this group was renamed to "teach consumers that protein is available in a variety of foods."[441] If the goal of renaming the former "meat & beans" group was to educate U.S. consumers about the presence of protein in a variety of foods, then dairy, an important source of high-quality protein, would be included in the protein group.

Furthermore, if consumers do not understand what protein is, knowing that different foods contain it is unlikely to be helpful in selecting a healthful diet. Qualitative research used in the development of the 2005 MyPyramid found that consumers had difficulty understanding the differences between nutrients.[439] In focus groups, consumers acknowledged that they did not understand the difference between saturated and unsaturated fats but did understand the difference between "solid fats" and "oils," which refer more directly to foods.[439] Therefore, explaining nutrition using familiar food-based

terminology may be a more effective way for consumers to understand dietary recommendations. The ChooseMyPlate.gov website states that its intention is to "to help consumers build healthier diets" with "user-friendly nutrition information."[444] Yet, the use of "protein" to describe a group of foods instead of using food to describe a group of foods may actually make MyPlate less helpful.

Finally, referring to this group of foods by their primary macronutrient also downplays the 2015 DGA's recommendation to focus on foods, not nutrients, when planning a healthful diet.[44] Focusing on nutrients makes good nutrition more difficult for consumers to understand and implement.[439] Just as the dairy foods group includes a list of "non-dairy sources of calcium," a "meat & beans group" could easily have one or more pages describing "other sources of protein" besides meat and beans, such as fish, eggs, seafood, and soy products.

Conclusion

Separating dairy from the rest of the "protein foods" on MyPlate brings into question what exactly a "protein food" is, a topic the MyPlate website does not address. Dairy foods include all nine essential amino acids and contain highly bioavailable and digestible protein, making them "protein foods," too. The "protein foods" group of MyPlate needs food-based name. Changing the name of the "protein foods" group to reflect the foods it contains may make it easier to communicate information about proper eating habits to a wider audience with a lack of knowledge about macronutrients. To improve the efficacy of MyPlate as a tool for consumer education, the information it

communicates needs to be both evidence-based and easy for both professionals and lay audiences to understand. The former name for the “protein foods” group, the “meat & beans” group would be an excellent place to start.

Table 9-1. Micronutrient differences between dairy foods and "protein group" foods

Nutrients	Micronutrients per 100 g[6]		
	Milk[******]	**Beef**[††††††]	**Beans**[‡‡‡‡‡‡]
Calcium, mg	125	13	73
Iron, mg	0.03	2.71	2.99
Magnesium, mg	11	22	51
Niacin, mg	0.1	5.66	0.113
Potassium, mg	150	333	454
Riboflavin, mg	0.19	0.176	0.037
Thiamin, mg	0.02	0.04	0.1
Vitamin A, IU	196	9	0
Vitamin D, IU	48	2	0
Vitamin E,	0.01	0.12	0.79

[******] Milk: 1% milk fat, with added vitamin A and vitamin D.

[††††††] Beef: ground, 90% lean, 10% fat, patty, cooked, broiled.

[‡‡‡‡‡‡] Beans: white, mature seeds, canned.

mg			
Zinc, mg	0.42	6.37	1.12

Table 9-2. Protein quality of foods as indicated by PDCAAS values

Protein Source	**Protein Digestibility Corrected Amino Acid Score (non-truncated score)**
Egg	100 (118)[25]
Ground beef	92[25]
Milk	100 (121) [25]
Soy	91[25]
Wheat	42[25]

Table 9-3. Shift in "protein" group name in USDA food guides over time

Food Guide (Year)	**Name of "Protein" Group**
Hunt Buying Guide (1916)	Meats and Other Protein-Rich Food[438]
Stiebeling's Buying Guide (1930)	Three protein groups (the lean meat, poultry, and fish group, the dry mature beans, peas, and nuts group, and the egg group)[438]
The Basic Seven (1940)	Meat, poultry, fish, eggs, dried beans, peas, nuts[438]
The Basic Four (1958)	Meat Group[438]
The Hassle-Free Guide (1979)	Meat, poultry, fish, and beans[438]
The Food Guide Pyramid (1992)	Meat Poultry, Fish, Dry Beans, Eggs, and Nuts Group[438]
MyPyramid (2005)	Meat and Beans[441]
MyPlate (2011)	Protein[3]

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Appendices

Appendix A: Participant Screening Questionnaire

Yes/No

Do you smoke or chew tobacco now?

For women, are you currently pregnant or lactating?

For women, is your menstrual cycle regular/consistent?

Have you taken antibiotics within the last 3 months?

Are you a vegetarian?

Are you lactose intolerant?

Do you have any food allergies?

If YES, what are they?

(Only an exclusionary criteria if likely to be found in test meals or pizza, i.e. wheat, dairy, fruit lentils, etc)

How many days per week do you usually consume breakfast?

_____ Days

(exclude if 3 days or fewer)

How many days per week do you usually consume lunch?

_____ Days

(exclude if 3 days or fewer)

Have you ever been diagnosed with the following diseases or conditions?

	YES	NO
Diabetes (type 1 or 2)	____	____
Heart disease	____	____
Kidney/Liver disease	____	____
Gluten intolerance	____	____
Cancer	____	____
Eating disorder	____	____
Crohn's Disease/Ulcerative Colitis	____	____
Diverticulitis	____	____
Any other gastrointestinal conditions	____	____

Have you ever had any gastrointestinal conditions or surgeries? ____ ____

If so, what? _____

(appendectomy, cholecystectomy or cesarean section okay)

Are you taking any medications for the following?

	YES	NO
Blood sugar	____	____
Cholesterol		

____ ____

Blood Pressure

____ ____

Weight loss

____ ____

Laxatives

____ ____

Anti-diarrhea

____ ____

Have you lost or gained more than 10 pounds in the past 3 months?

Do you participate in regular vigorous physical activity such as marathons, endurance bike races or triathlons?

How would you rate your present state of health compared to other people about your age?

Excellent ____ Good ____ Fair ____ Poor ____ (poor excluded)

Have you recently participated in a dietary intervention research study within the last month?

This study requires you to make dietary changes for a total of 20 days. Are you willing to do that?

This study requires you to consume mushrooms and beef. Are you willing to do that?

This study also requires you to collect and submit fecal samples to the lab. Are you willing to do that?

YES/NO

Are you planning on living in the Twin Cities area for the next 6 months?

If no, are you willing to travel to the Twin Cities for the study?

Do you travel outside the Twin Cities area frequently?
If so, what dates will you be out of town for an extended period of time?

Do you eat any of the following foods on MOST DAYS of the WEEK? If you only eat these foods occasionally please answer NO to each category. (Exclusionary only if subject eats a total of <u>three or more servings of the following foods on most days of the week</u>)

Yes **No**

High-fiber cereals (All Bran, Fiber One, Raisin Bran, etc.) ____ ____

High- fiber bars (fiber One, Kellogg's Fiber Plus, LUNA fiber) ____ ____

High-fiber bread products (100% whole wheat bread, bagels, pasta) ____ ___

Beans (black, kidney, pinto, white, etc.) ____ ___

High-fiber grains (barley, quinoa, buckwheat, spelt, etc) ____ ___

High-fiber fruits and vegetables

(> 1 cup berries, apples, pears, dried fruits, peas, beets, artichokes) ____ ___

Do you take any supplements? This includes vitamin/mineral supplements or multivitamins, fiber supplements like Metamucil or Citrucel, or herbal supplements, etc. (only exclusionary if subject takes more than three of recommended fiber servings).

____ ____

If yes, what:

Supplement: Dose/Frequency:

Are you currently consuming any probiotic yogurts or supplements? ____ ___

Description data collection only- not exclusionary.

Do you consume alcohol? ____ ____

If YES, how many drinks per week do you typically consume? (One drink = 12 oz beer, 4 oz wine, 1 oz hard liquor): _____drinks

Now I'm going to give you a short questionnaire about your eating patterns. Please respond with the answer that applies to you on most eating occasions.		Score
1. When I have eaten my quota of calories, I am usually good about not eating any more	T (+1) F	
2. I deliberately take small helpings as a means of controlling my weight	T (+1) F	
3. Live is too short to worry about dieting	T F (+1)	
4. I have a pretty good idea of the number of calories in common food	T (+1) F	
5. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it	T (+1) F	
6. I enjoy eating too much to spoil it by	T F	

counting calories or watching my weight	(+1)	
7. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat	T (+1) F	
8. I consciously hold back at meals in order to not gain weight	T (+1) F	
9. I eat anything I want, any time I want	T F (+1)	
10. I count calories as a conscious means of controlling my weight	T (+1) F	
11. I do not eat some foods because they make me fat	T (+1) F	
12. I pay a great deal of attention to changes in my figure	T (+1) F	
13. How often are you dieting in a conscious effort to control your weight? Rarely Sometimes Usually (+1) Always (+1)		
14. Would a weight fluctuation of 5 lbs affect the way you live your life? Not at all Slightly Moderately (+1) Very Much (+1)		

15. Do your feelings of guilt about overeating help you to control your food intake? Never Rarely Often (+1) Always (+1)	
16. How conscious are you of what you are eating? Not at all Slightly Moderately (+1) Very Much (+1)	
17. How frequently do you avoid “stocking up” on tempting food?\ Almost never Seldom Usually (+1) Almost always (+1)	
18. How likely are you to shop for low calorie foods? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)	
19. How likely are you to consciously eat slowly in order to cut down on how much you eat? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)	
20. How likely are you to consciously eat less than you want? Unlikely Slightly likely Moderately likely (+1) Very likely (+1)	

21. On a scale of 0 to 5, where 0 means no restraint in eating and 5 means total restraint what number would you give yourself? (0) Eat whatever you want, whenever you want it (1) Usually eat whatever you want, whenever you want it (2) Often eat whatever you want, whenever you want it (3) Often limit food intake but often "give in" (+1) (4) Usually limit food intake, rarely "give in" (+1) (5) Constantly limiting food intake, never "giving in" (+1)	
Total Score	
Exclude if score 11 or higher	

Appendix B: Mushroom Study Consent Form

MUSHROOM EFFECTS ON SATIETY AND GUT HEALTH MARKERS

CONSENT FORM

You are invited to participate in a research study of mushrooms and their effects on satiety and gut health. You were selected as a possible participant because you are a man or woman in good health.

We ask that you read this form and ask any questions you may have before agreeing to be in the study.

This study is being conducted by Joanne Slavin, Ph.D., RD in the Department of Food Science and Nutrition at the University of Minnesota. It is funded by the Mushroom Council.

Study Purpose

The purpose of the study is is to assess the effects of mushrooms on hunger, fullness, gut health, and the gut microbiome. The foods you will consume during the study are commonly consumed and are safe to consume.

Study Procedures

If you agree to participate in this study, we would ask you to do the following: attend two in-person visits for three hours each, consume provided foods for two ten-day periods, and collect and transport all fecal samples produced for two five-day periods to McNeal Hall 157 on the University of Minnesota's St. Paul campus.

At each in-person visit, you will be given a breakfast meal with or without mushrooms. These breakfast meals will be given in a random order. You will also be asked to complete a survey about your level of hunger before the meal and throughout the 3 hours following the meal. At each visit, you will also be asked to complete a breath hydrogen test twice following the meal. After the end of the first visit, you will be given a folder with gastrointestinal surveys and diet diaries. You will need to complete three surveys and three 24-hour diet diaries for each 10-day period following an in-person visit.

After each in-person session, you will also be given specific foods to incorporate into your diet for the dinner meal on the day of the in-person visit and for breakfast and dinner of the following nine days. You will also be given equipment for collection and transportation of all of your fecal samples to the lab for days six to ten of each ten day period. You will need to keep fecal samples cold using provided equipment and bring them to McNeal Hall 157 as soon as possible. When bringing fecal samples to the lab on

day 10 of the second ten-day period, you will return your completed folder to McNeal Hall 152.

After the first in-person visit, your second in-person visit will be scheduled at least 2 weeks later for a total of two three-hour in-person visits. You will need to bring your folder to the second in-person session.

Please see the attached study design graphic on the last page for an overview of study procedures.

Risks of Study Participation

The study has the following risks: Due to the nature of the fecal samples we are requesting, illness may result from unsanitary conditions of fecal collection. Hand washing before and after fecal collection is imperative.

The foods used in this study are provided in amounts commonly taken in foods. The breakfast foods used in this study are already foods available in the United States. There are no known side effects in the amounts used in this study.

Benefits of Study Participation

There is no guarantee that you will receive any benefit by participating in this study.

Study Costs/Compensation

Study related visits, procedures, and the food for the study will be provided at no cost to you.

Successful completion of each in-person session results in $50 per visit (total of two sessions).

Consumption of all study foods, successful completion and delivery of all diet records and tolerance questionnaires, and delivery of five day fecal collections results in payment of $200 per treatment period (total of two treatment periods).

A total compensation of $500 for completion of both scheduled visits, turning in all diet records and tolerance questionnaires, consumption of study foods, and submission of fecal samples will be provided. The principal investigator of this study is paid to cover the costs of conducting the research.

Research Related Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you

think that you have suffered a research related injury, let the study researchers know right away.

Confidentiality

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, be reviewed by departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute. Study data will be encrypted according to current University policy for protection of confidentiality.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University of Minnesota. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions

The researchers conducting this study are Joanne Slavin and Julie Hess. You may ask any questions you have now, or if you have questions later, **you are encouraged to** contact Julie at jmhess@umn.edu or at 612-625-5264 (office) or Dr. Slavin at jslavin@umn.edu or at (612)624-7234.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You

may also contact this office in writing or in person at *Fairview Research Administration, 2344 Energy Park Drive, St. Paul, MN 55108.*

You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Signature of Subject_____

Date_____

Signature of Person Obtaining

Consent_____

Date_____

Appendix C: VAS Satiety Assessment

How hungry do you feel?

I have never been more hungry _____ I am not hungry at all

How satisfied do you feel?

I am completely empty _____ I cannot eat another bite

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

A lot _____ Nothing at all

Appendix D: Food Portion Reference Guide

Appendix E: Bristol Stool Chart

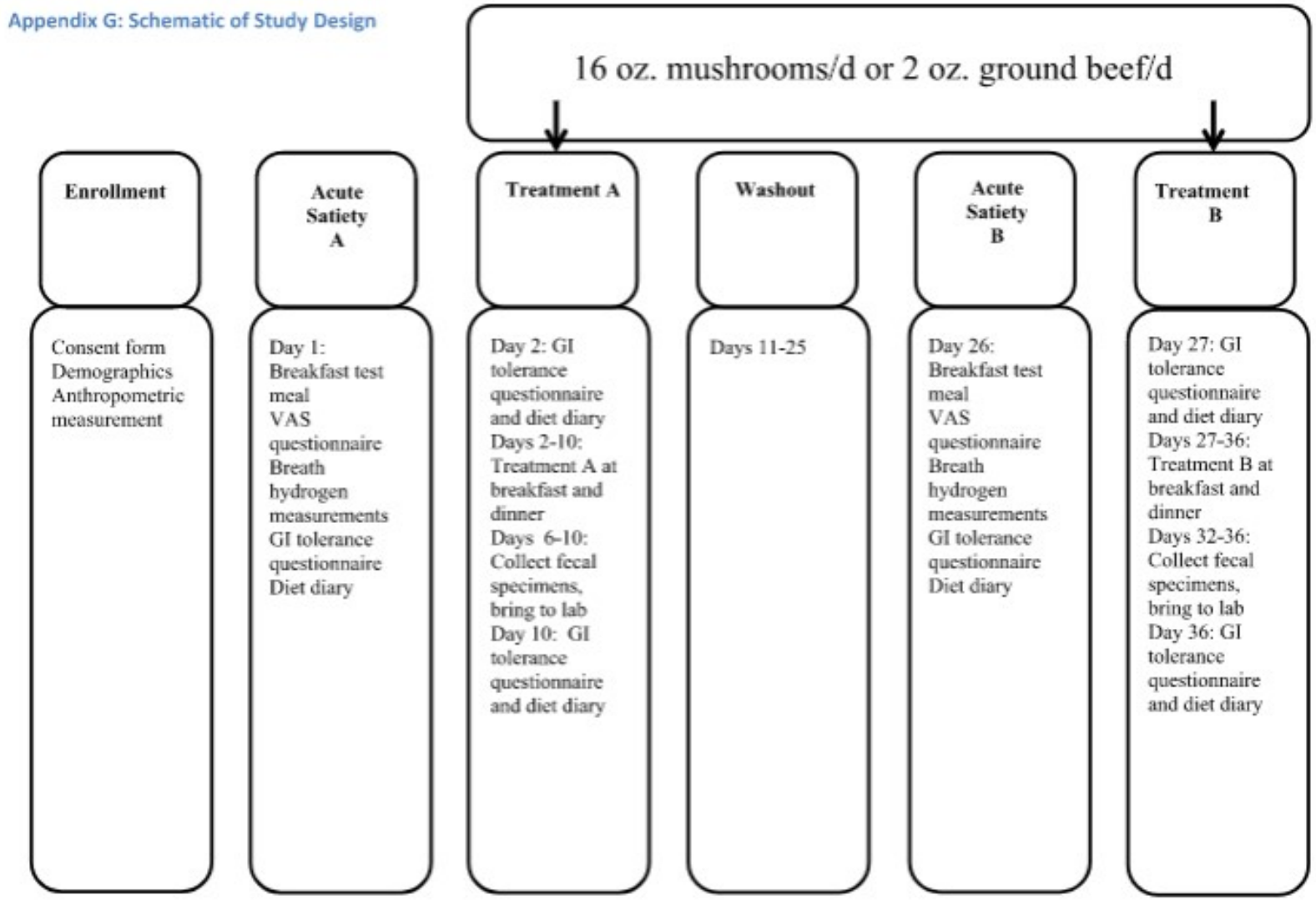
Bristol stool chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces, **Entirely liquid**

Appendix F: Gastrointestinal Tolerance Questionnaire

Please rate the level of the following symptoms you have experienced on the scale below.
Time: 0

1. Gas or bloating	☐ None	☐ Mild	☐ Moderate	☐ Severe
2. Nausea	☐ None	☐ Mild	☐ Moderate	☐ Severe
3. Flatulence	☐ None	☐ Mild	☐ Moderate	☐ Severe
4. Diarrhea or loose stools	☐ None	☐ Mild	☐ Moderate	☐ Severe
5. Constipation	☐ None	☐ Mild	☐ Moderate	☐ Severe
6. Gastrointestinal cramping	☐ None	☐ Mild	☐ Moderate	☐ Severe
7. Gastrointestinal rumbling	☐ None	☐ Mild	☐ Moderate	☐ Severe


16 oz. mushrooms/d or 2 oz. ground beef/d
Enrollment
Consent form
Demographics
Anthropometric measurement
Acute Satiety A
Day 1:
Breakfast test meal
VAS questionnaire
Breath hydrogen measurements
GI tolerance questionnaire
Diet diary
Treatment A
Day 2: GI tolerance questionnaire and diet diary
Days 2-10: Treatment A at breakfast and dinner
Days 6-10: Collect fecal specimens, bring to lab
Day 10: GI tolerance questionnaire and diet diary
Washout
Days 11-25
Acute Satiety B
Day 26:
Breakfast test meal
VAS questionnaire
Breath hydrogen measurements
GI tolerance questionnaire
Diet diary
Treatment B
Day 27: GI tolerance questionnaire and diet diary
Days 27-36: Treatment B at breakfast and dinner
Days 32-36: Collect fecal specimens, bring to lab
Day 36: GI tolerance questionnaire and diet diary